RESEARCHES ON TESTING CERTAIN BIO PREPARATES IN ORDER TO PREVENT BACTERIAN CANCER OF THE GRAPEVINE IN THE ODOBESTI VINEYARD

CERCETĂRI PRIVIND TESTAREA UNOR BIOPREPARATE ÎN VEDEREA PREVENIRII CANCERULUI BACTERIAN LA VIŢĂ DE VIE ÎN PODGORIA ODOBEŞTI

PODOSU-CRISTESCU Aurelia[1], MIHU G.[1], PAMFIL Maria[2], OPREA Maria[3]

[1]Research and Development Station for Viticulture and Oenology Odobesti, Romania

[2]I.N.C.D.C.F. Bucharest, Romania

[3]Research-Development Institute for Plant Protection, Bucharest, Romania

Abstract.*Under the pedological and climatical conditons of the Odobesti vineyard, the attack of the pathogenic bacterium Agrobacterium tumefaciens is manifested on the wooden parts of the vine (cords, stems) through the apparition on the surface of the organs of tumors of various sizes, covered in a characteristic spongious tissue. Generally, tumors form on the grafting point, in the areas where cords have been damaged (twisting, hail, breaking, etc). Using biological means in the prevention and control of diseases in cultivated plants is one of the issues in the attempt of finding ways to reduce both damage done by pathogens and pollution of the environment. In the attempts to limit and prevent tumorigenesis caused by Agrobacterium tumefaciens in grapevine, the eficacy of certain biological products based on Bacillus subtilis strains has been tested. The B4 biological product has inhibited the development of colonies in the highest percentage, respectively 95% in the table variety Muscat d' Adda, protecting the wounds of the vine from the attack of the Agrobacterium tumefaciens pathogen. The BSO biological product has manifested a moderate action in protecting the wounds against the action of the pathogen, with an inhibitory percent of up to 80%.*

Key words: *Agrobacterium tumefaciens*, tumors, biological products.

Rezumat. *În condiţiile pedo-climatice ale podgoriei Odobeşti, atacul produs de bacteria patogenă Agrobacterium tumefaciens se manifesta pe elementele lemnoase ale butucului (coarde, cordoane, tulpini, etc.) prin aparitia la suprafata organelor a unor tumori de dimensiuni variabile, acoperite cu un tesut buretos specific. In general, tumorile se formeaza la punctul de altoire, in zonele in care coardele au fost ranite (prin rasucire, grindina, rupere, etc). Utilizarea mijloacelor biologice în prevenirea şi combaterea bolilor la plantele de cultură se înscrie în preocupările găsirii căilor de reducere a pagubelor produse de patogeni, precum şi a poluării mediul înconjurător. În încercările de limitare şi prevenire a tumorogenezei produsă de Agrobacterium tumefaciens la viţa de vie, s-a testat eficacitatea unor produse biologice pe baza unor tulpini de Bacillus subtilis, relizate la INCDCF Bucureşti. Produsul biologic B4 a inhibat dezvoltarea coloniilor in procentul cel mai mare, respectiv 95%, la soiul de masa Muscat de Adda, protejand ranile plantelor de vita de vie de atacul produs de patogenul Agrobacterium tumefaciens. Produsul biologic BSO a manifestat o acţiune mijlocie in protejarea rănilor de aţiunea patogenului,*

procentul de inhibiţie fiind de pana la 80%.
Cuvinte cheie : *Agrobacterium tumefaciens*, tumori, produse biologice;

INTRODUCTION

Using biological means of disease prevention and control in cultivated plants is among the main concerns in the attempt to find ways to reduce damage done by pathogens and pollution of the environment. Putting into practice these biological means led to an increase in the study of microbe antagonism; helping to disperse antagonists leads to a limitation of their host's pathogens, diminishing loss (Josifović., 1967; Solovei, I.1974; Garett, C.M.E, 1979; Grindrat D., 1983; Ponchet, J., 1983; Severin V., Iliescu C.H., 2006; Zang W., Saks E.J., Lewis Ivev M.I., Miller S.A., Franus D.M., 2005).

Concerns in this fields are linked to the inhibitory action of microorganisms such as fungi (*Penicillium, Aspergillus, Chaetonium)*and bacteria*(Bacillus sp., Pseudomonas sp. Xanthomonas sp., Erwinia amylovora),* on the pathogenous bacterium *Agrobacterium tumefaciens.* Thus, a good rate of prevention in tumor appearance has been observed after using avirulant *Agrobacterium* strains, which have prevented the infection of the cultivated plants in the field, by excluding virulant strains from the penetration spot (the HLB-2 strain, of the biovar 1, isolated by Xiaoyng în China(8). The treatment of the grafted cuttings, before planting in the nursery, using a biological product derived from HLB-2, of the biovar 1, isolated by Xiaoyng în China,has inhibited cancer in high percentages (Pu et Goodman, 1993). Good results have been obtained with a product obtained from a strain of *Agrobacterium,* patented as a preparation with the name Biozin, by Zinca, 1971.

MATERIAL AND METHOD

I.1. Isolating and identifying the bacterium

In the bleeding phenological phase of the grapevine crude sap was collected by attaching sterile test tubes to the cords of plants from the Black Feteasca variety which had shown symptoms of bacterial cancer. The collected liquid has been included in a colony environment (peptone, meat juice, glucose-agar) and distributed in Petri dishes. Six days later, the surfaces developed colonies characteristic for *Agrobacterium tumefaciens*.

I.2. Testing the protective capacity

During the experiment the response reaction has been tested on a number of five varieties, as follows: Chasselas doré, Muscat d' Adda, Muscat Hamburg, Royal Feteasca si Black Feteasca.

Cords taken from healthy plants had been cut at 3 buds and placed in water containers at constant temperature in order to start the vegetation process. The inoculation has been done with the *Agrobacterium tumefaciens* we have isolated.

The biological products used for testing the protective capacity have been produced by the National Institute for Chemical Pharmaceutical Research and Development in Bucharest (INCDCF), and are strains of *Bacillus subtilis* , as follows:

- BSP + BSU – isolated from twitch and dried cereals

--BSP + BSV – isolated from twitch and medicinal plants

-B4-isolated from leaves in decidous forests (immediately after cutting when the wounds were fresh)

Testing has been conducted under laboratory conditions, thus, on cords taken from

different varieties of the Odobesti vineyard, cotton balls soaked in a suspension of bacterial cells (active substance 10^7 ufc/ml) have been applied. The virulant cells suspension has been administered simultaneously with the biological product from INCDCF Bucharest.

In order to establish whether the wound had been protected by the action of the pathogen, after 25 days of treatment, one cm samples have been taken from the place of the infection up to the first bud, and the fragments distributed on the culture environment characteristic for the growth of *Agrobacterium tumefaciens*,and observations have been made after 6 days.

RESULTS AND DISCUSSIONS

Following the investigations conducted in the grapevine plantations of SCDVV Odobeşti, Vrancea, concerning the varieties' reaction to the attack of *Agrobacterium tumefaciens,*it has been observed that the table grapes varieties are more sensitive than the wine grapes, and of these, the Feteasca regala variety has considerable resistence.

Fig.1. Colonies of the *Agrobacterium tumefaciens* bacterium

Under laboratory conditions colonies of the pathogen bacteria *Agrobacterium tumefaciens* have been obtained, isolated in the liquid leaked by the plant in the bleeding phenological phase. On a colony environment, (peptone, meat juice, glucose-agar), *Agrobacterium tumefaciens* has formed white-yellow colonies with a creamy aspect and brownish-yellow reverse (fig.1).

Table 1

The frequency of grapevine plants affected by cancer (*Agrobacterium tumefaciens*) in a grapevine plantation of the Odobesti vineyard, 2008

Variety	Analyzed plants	Diseased plants	Attack frequency (%)
Muscat Hamburg	58	49	84,48
Muscat d' Adda	55	44	80,00
Chasselas dore	50	38	76,00
Feteasca regala	61	36	59,02

Following the administration of biological products of fresh wounds, simultaneously with the inoculation of *Agrobacterium tumefaciens,* compared to control plants which had not been infected nor treated, the fresh wounds on the cords have been protected by the pathogenic action of the *Agrobacterium tumefaciens*, in a percentage between 70-95%, depending on the strain used for

the preparation of the biological product (table2).

The B4 biological product has noticeably protected the freshly cut wounds from infections with *Agrobacterium tumefacien*, with an inhibition percentage of 80-95% in all observed varieties.

The BSV product has protected the wounds of the cords from the pathogenic action of the bacterium in a percentage of 75-84%.

The BSO biological product has had a weaker effect of protecting wounds from cutting, the percentage being of 60-70%. (fig. 2)

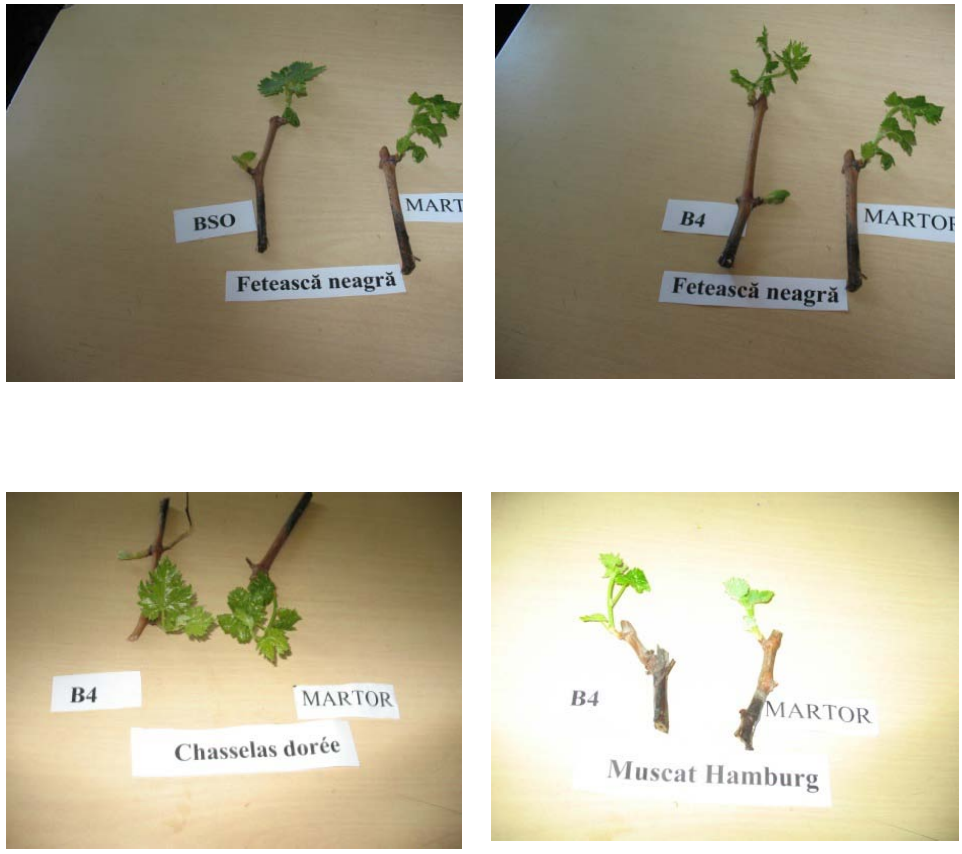

Fig. 2. The eficiency of biological products in protecting wounds against the pathogenic action of *Agrobacterium tumefaciens*

The table varieties (table 2) Chasselas dore, Muscat d' Adda and Muscat Hamburg have manifested an increased sensibility for the pathogenic bacterium. Among the wine varieties, the Royal Feteasca may be considered resistant to the action of the pathogen.

Table 2

The growth of colonies and the inhibition percentage of the bioproducts tested on grapevine varieties for table grapes

Nr. crt.	Variety	Product	Conc. (%)	Colonies grown in Petri dishes (no.)	Inhibition %
1	Muscat d' Adda	BSO	5	30	70
		BSV	5	25	75
		B4	5	5	95
		Control plant	-	100	0
2	Chasselas doré	BSO	5	40	60
		BSV	5	16	84
		B4	5	10	90
		Control plant	-	100	0
3	Muscat Hamburg	BSO	5	20	80
		BSV	5	16	84
		B4	5	10	90
		Control plant	-	100	0
4	Feteasc㠠neagra	BSO	5	23	77
		BSV	5	19	81
		B4	5	10	90
		Control plant	-	100	0
5	Feteasă regala	BSO	5	20	80
		BSV	5	30	70
		B4	5	10	90
		Control plant	-	85	15

CONCLUSIONS

1. The B4 biological product has inhibited the growth of colonies in a percentage of 90 - 95%, in the Muscat de Adda table variety, significantly protecting the wounds of the grapevine plants against the attack of the *Agrobacterium tumefaciens* pathogen.
2. The biological product BSO has had moderate effects in protecting the wounds against the action of the pathogen, with an inhibition percentage of up to 80%.

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