BIOLOGICAL SOLUTIONS TO PREVENT AND DECREASE THE ATTACK PHYLLOXERA (*PHYLLOXERA VASTATRIX*)

SOLUȚII BIOLOGICE DE PREVENIRE ŞI DIMINUARE A ATACULUI DE FILOXERA (*PHYLLOXERA VASTATRIX*)

***FÎCIU Lidia*[1], *DEJEU L.*[1]**
e-mail: ficiulidia@yahoo.com

Abstract. *To prevent and decrease the attack of phylloxera was tested a technology based on amplifying the suppressive properties of soil on phylloxera attack by improving the fertility and biological activity of the soil. Biopreparats with Beauveria sp. fungi have been used in granular form on organic support (grains of wheat and barley) as an active substance for Beauveria. The organic fertilizer such as manure and compost introduced in soil represent the support used for fungal strains multiplication and spread. The results obtained showed that the symptoms produced by the phylloxera on roots, are more numerous in the case of compost (20 t/ha) used as organic supports compared to the manure (20 t/ha), mixed with 50 kg/ha biopreparat administered at a depth greater than 20 cm. In the case we used a quantity of 20 t/ha manure mixed with 200 kg/ha biopreparat introduced in soil at 10 cm depth, the frequency of symptoms has been greatly reduced.*
Key words: viticulture, phylloxera, suppressive effects

Rezumat. *Pentru prevenirea şi diminuarea atacului de filoxera s-a testat o tehnologie de amplificare a însuşirilor represive ale solurilor asupra filoxerei prin sporirea fertilității si activității lor biologice. S-au folosit biopreparate sub formă de granule pe suport organic (boabe de grâu şi orz), care au ca substanță activă fungi de Beauveria sp. Produsele organice de fertilizare mranița şi compostul introduse în sol reprezintă substraturile pe care tulpinile fungice le exploatează în procesul de multiplicare si conidiogeneză, asigurând răspândirea patogenului. Din observațiile făcute s-a constatat că simptomele produse de filoxera pe rădăcini, sunt mult mai numeroase la variantele în care s-a utilizat compostul (20 t/ha) ca suport organic în amestec cu 50 kg/ha biopreparat administrat la o adâncime de 40 cm, față de variantele unde s-a folosit ca suport organic mranița (20 t/ha). În cazul utilizării unei cantități de 20 t/ha mraniță în amestec cu 200 kg/ha biopreparat, administrat la 20 cm adâncime, frecvența simptomelor a fost mult diminuată.*
Cuvinte cheie: viticultură, filoxera, efect represiv

INTRODUCTION

The phylloxera was discovered in 1868, its attack causing the biggest disaster know (Bazille et al., 1868).

[1] University of Agricultural Sciences and Veterinary Medicine Bucharest, România

In Romania, as well as worldwide, phylloxera is the most important pest in the vineyard, for which there aren't the effective methods of control, but only to avoid the damage.

Climate change (warm winters, high temperatures, low rainfall), cultural techniques of non-compliant (planting too deeply rooted grafts) will facilities appearance of some strains with a high degree of pest and enhancing the area of spread of the pest (Ray, 2006; Sabbour and Abbass, 2006). From observations and determinations made to found an increase in aggressiveness and virulence, an increase in the number of generations and a worrying attack of galicole form a growing number of *vinifera* varieties (Jeffrey and Andrew Walker, 2001).

Beauveria genus as of fact and other genus of fungal entomopathogenic for good growth broadcasting and spore is in need of the organic matter. It is also one of the reasons for which fertile soils, rich in organic matter have a suppressive action more than the poor soils, unstructured (Sabbour and Abbass, 2006).

MATERIAL AND METHOD

For this was realized an experimental device located in vine school from ICDVV Valea Calugareasca where they been planted seedlings of vines for rooting of 38 +/-2 cm of Merlot. The experimental variables were the organic fertilizer and dose of biopreparat. Of their combination resulted 12 variants:

The factor A – vinifera variety - a_1 -Merlot

The factor B – the type of fertilizer - b_1 - manure
- b_2 - compost
- b_3 - chemical fertilizer
- b_4 - unfertilized

The factor C – the quantity of biopreparat - c_1 - 50 kg/ha
- c_2 - 100 kf/ha
- c_3 - 200 kg/ha

The factor D – the depth by incorporation of the mixture biopreparat –fertilizer
- d_1 – 10 cm
- d_2 – 20 cm

The planting was done in simple billon, rich microbiological fertilizer management (calculated per unit of area) has realized with the cutting billon. By the planting in the field to follow the behaviour in natural conditions of biopreparat which contains strains of *Beauveria bassiana*, and on the other the cumulative effect of organic fertilizer combination x biopreparat x application rate on phylloxera radicicola form.

After 60 days after planting have been taken the soil samples from three points on each variant, at two depths (0-2 and 2-10 cm) after which have been mixed.

In order to quantify the number of colonies, the evaluation of vegetative growth was achieved after about a week of incubation at a temperature of 26 ± 1 °C in the dark.

Counting the colonies has been done using the numerator of Colony Star.

The dates were analyzed using ANOVA test and comparisons between test environments were made using the ANOVA test (ONE WAY and Linear Regression) ($p < 0.05$) using software Bio Stat 2008.

The repressive potential was achieved by quantifying the symptoms produced by the phylloxera radicicola form on roots using a scale from 1-4 (table 1).

Table1

Scale for quantifying the symptoms produced by the *Phylloxera radicicola*

Note	Root aspect	Nodosities		Phylloxera (adults, larvae, eggs, colony)
		Dimensione	Number	
1	Not curved	Absent	Absent	Absent
2	Low curved	Small	Fiew	Present in small numbers
3	Curved	Middle	Present	Present medium
4	Very curved	Sea	Many	Colony

For each note was assigned a color. It has been analyzed 5 roots of approximately 10 cm in length and with a diameter of approximately 3-5 mm.

RESULTS AND DISCUSSION

From the observations that had purpose verifying the behaviour pathogenic strains (table 2) showed that the strains of *Beauveria bassiana*, after a new isolation from organic fertilizers use as nutrient substrates for fungal multiplication shall their viability and virulence.

Table2

Biological parameters of strains of *Beauveria bassiana* after a new isolation of organic fertilizers use as substrates for growing nutrient from fungal contamination

Fungal strain	Vegetative growth/CGA (after 15 days from seeding)		Conydiogenesis (Conidy number x10^{10}/g)	Viability germination (%)
	medium size colonies (number)	average daily growth rate (number)		
Nutrient substrate: compost				
Beauveria bassiana	5,7	0,32	8,6	97
Nutrient substrate: manure				
Beauveria bassiana	6,1	0,4	9,2	99
Standard: nutrient substrate synthetic culture medium (peptona-dextroza-agar)				
Beauveria bassiana	5,9	0,38	2,9 (x10^{10}/cm^2)	100

Following the analysis of soil samples taken from the experimental variants and passing the results by statistical filter (table 3) that there is a significant difference between organic use fertilizer (manure, chemical fertilizer, compost, organic without support) and no between doses of biopreparat.

Table3

Results concerning the establishment of organic substrate and of the doses of application of the biopreparat in natural conditions

Variant	Organic support	*Beauveria bassiana* dose kg/ha	Number of colonies x 10^4/ g sol
1	Manure	50	9±1,27
2	Manure	100	20,9±0,05
3	Manure	200	22,8±0,02
4	Compost	50	22±1,14
5	Compost	100	20±0,04
6	Compost	200	81,4±0,21
7	Chemical fertilizer	50	10±1,2
8	Chemical fertilizer	100	14±0,07
9	Chemical fertilizer	200	7±0,08
10	Without organic support	50	16,5±0,18
11	Without organic support	100	37,2±0,25
12	Without organic support	200	18±1,13

As a conclusion, on the basis of the results obtained, we can affirm that the number of the conidy in the ground of persistent field does not depend on the dosage of the biopreparat inoculated. Considering this fact, and the behaviour of the conidy of the *Beauveria bassiana* inoculated into the soil in experimental laboratory conditions, studied previously, we can estimate that the dose of 1, $48x10^{12}$ conidy/ha can ensure microbiological inoculation with *Beauveria bassiana.* To ensure this doses of active substance/ha, it is necessary a quantity of 50 up to 100 kg/ha biopreparat fungi that contain min. 2.7 g active substance/kg.

From the analysis of data concerning the frequency and severity of the attack of phylloxera radicicola form (table 4) by inoculating the soil of biopreparat based on *Beauveria bassiana* fungal strains is obtained an increase repressive effect against phylloxera radicicola form.

Table 4

Influence of combined action of fertilizer and biopreparat action on the symptoms produced by the phylloxera radicicola form

Dose/ha/ biopreparat based on strains of *Beauveria bassiana* fungal	Manure		Compost		Chemical fertilizer		Without fertilizer	
	Note	E%*	Note	E%*	Note	E%*	Note	E%*
50	3,6	36,8	5,7	8,6	5,4	6,3	6,2	5,0
100	3,0	47,4	3,2	43,6	3,2	44,8	4,5	31,8
200	2,6	54,3	3,6	58,6	3,8	34,5	4,2	36,4
Media	3,1	46,1	4,2	36,9	4,1	28,5	5,0	24,4
Without fertilizer and biopreparat (control)	5,7		6,2		5,8		6,6	

***** The efficiency

As we expected, using manure as organic support ensure a good growth, spread and the sporulation of fungal strains based on *Beauveria bassiana* and consequently increase the soil repressively on *Phylloxera radicicola* form. Average efficiency obtained (calculated from the control) was 46.1%.

When applying a dose of 50 kg biopreparat/ha, mixed with manure, the efficiency is 36.8%, 8.6% with the compost, and only 5% without fertilizer. By increasing the dose to 100 kg biopreparat/ha, respectively 200 kg biopreparat/ha, in addition, the best efficiency we find the mixture with the manure (47.4% and 54.3%). Applying a dose of 200 kg biopreparat /ha achieved an efficiency of 36.4% even the variants without organic support.

As regards the optimal depth of the incorporation of biopreparat-fertilizer mixture has found that by increasing the depth of the administration decrease repressive effects even if increasing the dose of the biopreparat, from 27.2% at a depth of 20 cm to 22.0% at a depth of 40 cm in the administration of 100 kg biopreparat/ha, respectively from 35.0% at a depth of 20 cm to 22.1% at a depth of 40 cm in the administration of 200 kg biopreparat/ha (table 5).

Table 5

Influence of the depth of biopreparat-fertilizer mixture

Biopreparat - fertilizer	Note	E % *
Manure 20 t/ha + 100 kg/ha biopreparat to 20 cm depth	5.6	27.2
Manure 20 t/ha + 100 kg/ha biopreparat la 40 cm depth	6.0	22.0
Manure 20 t/ha + 200 kg/ha biopreparat la 20 cm depth	5.0	35.0
Manure 20 t/ha + 200 kg/ha biopreparat la 40 cm depth	6.0	22.1
Without fertilier and withoutbiopreparat (control)	7.7	

* - The efficiency

CONCLUSIONS

1. The manure and compost, organic fertilizer products of the vine were colonized with strains of *Beauveria bassiana*, resulting microbiologically rich and fertilizer effect of increasing the repressive potential of the soil compared with phylloxera.

2. The manure and compost have provided the stability properties of pathogenity and the virulence of fungal strains. The contribution of the organic matter enriched microbiological favoured reducing the effects of phylloxera radicicola form.

3. The optimal dose of application of the biopreparat 20 t/ha manure and compost is 100 kg/ha.

4. The optimal depth of the incorporation of biopreparat-fertilizer mixture is 20 cm.

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