EFFECT OF DIFFERENT SOLID SUBSTRATES ON MASS PRODUCTION OF *BEAUVERIA BASSIANA*

STUDIUL EFECTULUI DIFERITELOR SUBSTRATE SOLIDE ASUPRA PRODUCȚIEI DE BIOMASĂ FUNGICĂ DE *BEAUVERIA BASSIANA*

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Abstract. *The objective of this study was to select an optimal culture system for the production of the Beauveria bassiana fungal biomass with high virulence, in order to realize a compound which will be used in the biological control of phylloxera galicola. The influence of different solid substrates on the production of Beauveria bassiana fungal biomass was analyzed using the diphasic liquid–solid fermentation technique. The liquid phase, represented by a spore suspension with fungitoxic effect of Beauveria bassiana, in an active growth phase, was mixed, in a ratio of 1/1 with the solid substrates represented by: barley, rice, broken maize and wheat. The best results were obtained on solid substrate represented by wheat which was selected for the production of fungal biomass. The worst results were obtained on broken maize. The selected culture substrat will be applied to ensure enough spores in order to achieve the compound which will be used in the vineyard.*
Key words: *viticulture, phylloxera, substrate*

Rezumat. *Studiul a urmărit selecția unui sistem de cultură optim pentru producerea de biomasă fungică de Beauveria bassiana cu virulență mare, pentru realizarea unui biopreparat ce va fi utilizat în combaterea biologică a filoxerei galicola. Influența diferitelor substraturi solide asupra producerii de biomasă fungică de Beauveria bassiana a fost analizată folosind tehnica de fermentație în sistem difazic: lichid–solid. Faza lichidă, reprezentată de o suspensie de spori de Beauveria bassiana, cu efect fungitoxic, aflată în faza de creştere activă a fost amestecată în proporție de 1/1 cu un substrat solid reprezentat de: orz, orez, mălai granular şi grâu. Cele mai bune rezultate au fost obținute pe substratul solid grâu, substrat selectat pentru obținerea biomasei fungice, iar cele mai slabe pe mălai granular. Substratul de cultură selectat va fi aplicat pentru a se asigura o cantitate suficientă de spori pentru realizarea biopreparatului fungicid ce va fi aplicat în plantație.*
Cuvinte cheie: *viticultură, filoxera, substrat*

INTRODUCTION

The application of the biological methods for the pest control represent an alternative to the chemical control. One of the most effective methods of the pest control applied in the last time is based on the using of the bio-fungi

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preparations based on the entomopathogenic fungus *Beauveria bassiana*. They act on the insect populations, belonging to various orders: Coleoptera (Liuel Bauer, 2008), Homoptere (Dara et al., 2007), and hemiptere (McGuire et Leland, 2006).

The production of the *Beauveria bassiana* stable conidia, with high stability and viability over time can be achieved by the application of the fermentation technology in difazic system: liquid-solid, in 1/1 ratio. This system consisits in the mixing of a suspension of blastospores in the liquid medium with a solid nutritional substrate.

The solid phase represents the support for the development of aerial conidia. The conidia produced by the fungus in this way can be directly used in the form of suspension or like apores powder obtained after filtration and drying .

This study aimed to establish a method that can ensure the production of *Beauveria bassiana* fungal biomass with high virulence in order to be used to perform the treatments in the vineyards.

MATERIAL AND METHOD

For the production of the fungal biomass, the technique of fermentation in difazic system: liquid-solid, developed within the LUBILOSA project was used (Lutte Biologique contre les Locustes et Sauteriaux, Lomer et al., 1997).

The scheme of the experiment is presented in Figure 1.

A liquid conditioned bio-product of *Beauveria bassiana* Bb01 (micelles and blastospori) was used in the experiment. The bio-product was in the active growth phase, on the medium having the following composition: lactose (20 g), peptone (20 g), crumbled *Tenebrio molitor* (0.5 g), sterile distilled water (1000 ml).

The bio-product has been obtained by The Research and Development Institute for Plant Protection, Bucharest and was maintained by repeated subcultivation every three months, in the sporulated form in the germplasm collection of The Reserch and Development Institute for Viticulture and Enology, Valea Calugareasca, on Potato Dextrose Agar medium, at 4°C.

The reactivation of the bio-product was achieved by the cultivation on Potato Dextrose Agar medium until a purity of 100% was achieved.

Four solid substrates: barley (a1), rice (a2), broken maize (a3) and wheat (a4). were used in the experiment. 250 g of each substrate were distributed into special bags and in Erlenmayer of 1000 ml. The rice was boiled gently until the beans became soft. The barley, broken maize and the wheat were wetted with sterile distilled water. The solid substrates were then sterilized by autoclaving at 121°C and 1 atm, for 30 minutes.

Three replicate were carried out for each treatment. The bags and Erlenmayer vessels containing the solid substrates were inoculated with a suspension of *Beauveria bassiana* (liquid phase) (ratio 1:1), were then shaken and incubated in a thermostat at a temperature of 25°C for the development of the fungal hyphae.

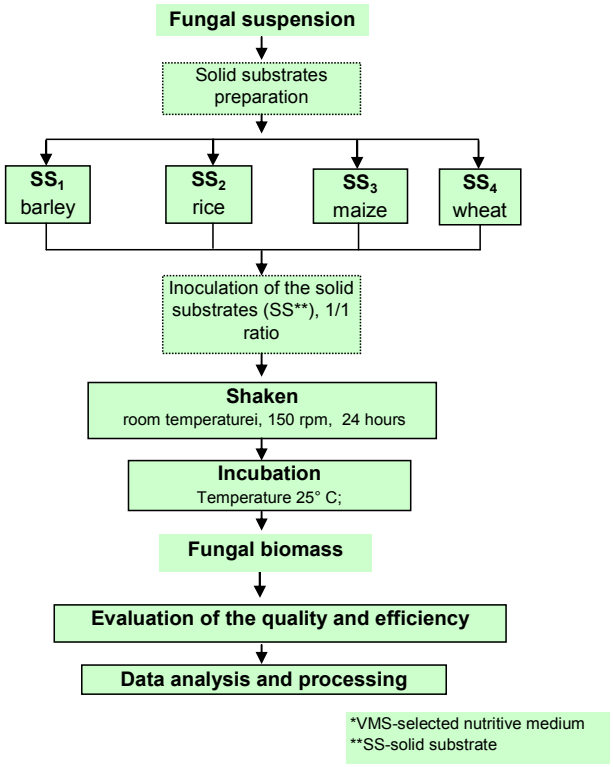

Fig. 1 - The scheme of the experiment

After 15 days of culturing, the substrates were aerated by the removing the plugs of wool, respectively by the opening of the the bags in order to stimulate the sporulation. The harvest of the spores developed in the liquid medium was performed after 22 days of culturing of the fungal suspension by the filtration of the fungal suspension through a piece of lint in order to retained and remove the micelles.

The selectivity of the conditioned bio-product of *Beauveria bassiana*, in the liquid form related to the nutritive substrate was determined on the basis of the following observations and measurements:

- the vegetative growth rate
- the ability for colonization
- the viability
- the insecticide titer (virulence)

The vegetative growth rate was determined by the analysis of the capacity of the growth of fungal mycelium per unit time, respectively 24 hours.

The colonization ability was established by the determination of the time required for the fungal mycelium to cover a certain amount of nutrient substrate - 100 grains in the case of the barley, rice and wheat substrates, and 100 g for broken maize.

The conidiogenesis represent the number of the conidia which was developed in a millilitre of the fungal suspension and was established by the decimal dilutions method. The determinations were made after 7, 12, 17 and 22 days of culturing.

The viability was determined by analyzing the percentage of the germination capacity of conidia by the method developed by Inglis et al., 1993.

The titer insecticide (virulence) was determined in the laboratory conditions on the larvae of phylloxera radicicola. The lethal concentration (DL_{50} – the dose which determine the lethal effect for 50% of the larvae tested) and the lethal time (TL_{50}) were determined.

The testing was performed by using the fungal suspensions with different concentrations of conidia: 0 ; 1 x 10^2 ; 1 x 10^4 ; 1 x 10^6 ; 1 x 10^8 ; 1 x 10^9 which were used for the treatment of 20 larvae of phylloxera galicola. After 2 hours of treatment, the larvae were placed on the growth medium. During 7 days, the observations regarding the larval mortality were made. For each concentration three repetitions were made. The experience was of monofactorial type. The results were statistically processed by analysis of variance based on the „XLSTAT" programme.

RESULTS AND DISCUSSIONS

The solid culture substrate significantly influenced the capacity of fungal mycelium to growth. There were very significant differences, in the positive way in case of the variant with wheat, the daily mycelium groth rate being 0.69 cm/day. The worst evolution has been observed in case of the utilization of the broken maize as a substrate, with a daily rate of the mycelium of 0.07 cm/day. The other two variants, respectively barley and rice culture, have determined a similar evolution in the development of the mycelium with an average capacity of growh of 0.31 and 0.35 cm, respectivelly.

The fungal strain showed the best ability to populate the solid substrate on wheat (100 grains were covered by the fungal mycelium on the average 19.33 hours) and barley (the full coverage of the grains lasted on average 27 hours), the differences being very significant in comparison with the media values of the observations. The lowest capacity of colonization (an average of 113.33 hours) was observed on the broken maize substrate (table 1).

Table 1

Influence of the substrate on the colonization ability of the fungal strain

Variant	The time required to cover 100 grains (hours), (average)	D +/-	Signification
a_1 - barley	27,00	-20,92	B
a_2 - rice	32,00	-15,92	b
a_3 – broken maize	113,33	65,41	A
a_4- wheat	19,33	-28,59	B
Average	47,92	0,00	
Control	47,92	0,00	
DL 5% = 8.45; DL 1.0 % = 12.80; DL 0.1 % = 20.57			

A, B: $p<0.01$; a, b: $p<0.05$ indicate the significance of the comparison in the same row

The observations concerning the number of conidia developed in one millilitre of fungal suspension after 22 days of culturing revealed the very significant differences in the positive way for the wheat variant, and in a negative way for rice and broken maize variants (Table 2, Figure 2).

Table 2

Influence of the substrate on the conidiogenesis

Variant	Conidia number x 10^9 (average)	D +/-	Signification
a_1 - barley	7,60	0,90	
a_2 - rice	4,90	-1,80	B
a_3 - broken maize	1,63	-5,07	B
a_4 - wheat	12,95	6,25	A
Average	6,70	0,00	
Control	6,70	0,00	
DL 5% = 0.66; DL 1.0 % = 1.00; DL 0.1 % = 1.60			

A, B: p<0.01; a, b: p<0.05 indicate the significance of the comparison in the same row

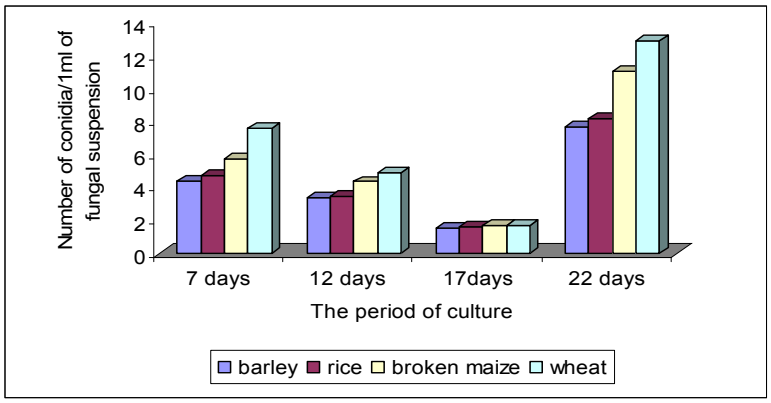

Fig. 2 - The dynamic of the conidiogenesis

The culture of the fungal suspension on wheat substrate has determined the best score of the spore viability (95%). The lethal concentration (DL_{50}) of the strain developed on the wheat (the best variant) was $7.0x10^6$ conidia/ml and the lethal time (TL_{50}) was 5 days. DL_{50} has the value of $5x10^8$ for barley and rice. For the broken maize a value greater than $5x10^8$ was registered (Figure 3).

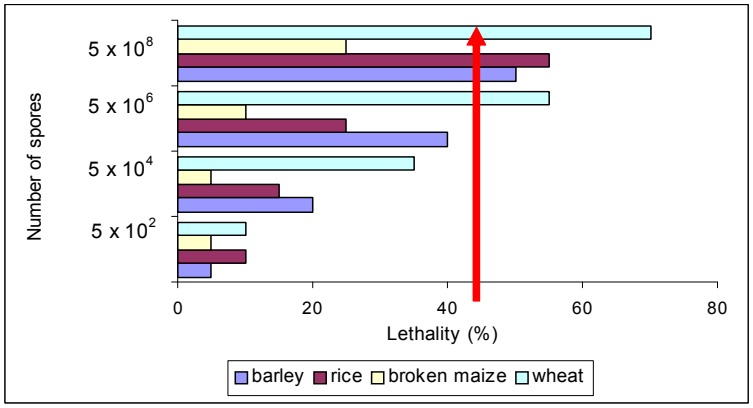

Fig. 3 - The lethal concentration of the strain depending on the nature of the solid substrate

CONCLUSIONS

1. The solid culture substrate significantly influenced the capacity of fungal mycelium to growth, the conidiogenesis and the viability of the spores.

2. The best results were obtained on the solid substrate represented by wheat, which was selected for the obtaining of the fungal biomass. The worst evolution has been observed in case of the utilization of the broken maize as a substrate.

3. The selected culture difazic system will be further applied in order to ensure a sufficient quantity of spores which will be used for the bio-fungi preparation that will be applied in the vineyard.

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