# INFLUENCE OF THE NUTRITIVE SUBSTRATES ON THE QUALITY OF THE *BEAUVERIA BASSIANA* SPORES

## INFLUENTA SUBSTRATURILOR NUTRITIVE ASUPRA CALITĂȚII SPORILOR DE *BEAUVERIA BASSIANA*

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***Abstract***. *In order to select an efficient culture medium for the multiplication of the fungal suspension with insecticidal activity was performed the analysis of the Beauveria bassiana spores in relation with the nutritive substrate. Six different carbon sources: sucrose, glucose, maltose, fructose, lactose, cellulose and three nitrogen sources: yeast extract, peptone and corn extract were tested. A conditioning liquid form, Beauveria bassiana (Bb01) with fungitoxic effect on phylloxera radicicola, which was obtained by The Research and Development Institute for Plant Protection, Bucharest. Among the six carbon sources tested, lactose was the best carbon source for the fungus multiplication and sporulation. Combinations of lactose with yeast extract and peptone resulted in a higher production of spores. The use of cellulose as a carbon source in combination with all three sources of nitrogen conducted to the worst results, both in terms of vegetative growth and the amount of spores produced.*
***Key words:*** *viticulture, phylloxera, substrate*

***Rezumat*** *În scopul selecției unui mediu de cultură eficient pentru multiplicarea suspensiei fungice cu activitate insecticidă a fost realizată analiza calității sporilor de Beauveria bassiana, în funcție de substratul nutritiv. Au fost testate 6 surse de carbon: zaharoza, glucoza, maltoza, fructoza, lactoza, celuloza şi 3 surse de azot: extract de drojdie, peptona şi extract de porumb. În experiment a fost utilizat un produs condiționat sub formă lichidă de Beauveria bassiana (Bb01) cu efect fungitoxic asupra filoxerei radicicola, obținută de Institutul de Cercetare-Dezvoltare pentru Protecția Plantelor, Bucureşti. Dintre cele şase surse de carbon testate, lactoza a fost cea mai bună sursă pentru multiplicare şi sporulare. Extractul de drojdie şi peptonă au determinat o producție mare de spori viabili numai în combinație cu lactoza. Cele mai slabe rezultate, atât din punct de vedere al creşterii vegetative cât şi al cantității de spori viabili produse, au fost obținute în condițiile utilizării celulozei ca sursă de carbon pentru toate cele trei surse de azot.*
**Cuvinte cheie**: *viticultură, filoxera, substrat*

## INTRODUCTION

The intensification of the vegetative production during the last decades is associated with the existence on the market of the effective and efficient plant protection products. These are synthetic inorganic and organic chemical

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substances which have a negative impact on the human healts, animals and environment. Among them, the chemical insecticides are less used as pest control agents due to the high cost of production and their high toxicity, persistence and accumulation in the environment.

The application of the biological methods for the pest control represent an alternative to the chemical control (Mc Coy et al.,1988). The using of the entomopathogenic fungus *Beauveria bassiana*, the „active substance" of the bio-fungi preparations in the biological control of the insect populations is one of the most effective ways of the pest control applied in the last time (Liuel Bauer, 2006; Dara et al., 2007).

In order to achieve the bio-fungi preparations it was necessary previously to determine the medium culture conditions which enshure the multiplication and sporulation of the *Beauveria bassiana* fungus while maintaining its insecticide properties.

This study aimed to analyse the quality of the *Beauveria bassiana* spores in relation with the nutritive substrate and the selection of the most efficient culture medium for the multiplication of the fungal suspension with insecticidal activity.

## MATERIAL AND METHOD

A conditioning liquid product of *Beauveria bassiana* (Bb01) with fungitoxic effect was used in the experiment. This product was obtained by The Research and Development Institute for Plant Protection, Bucharest and was maintained by repeated subculturing every three months in the germplasm collection of The Reserch and Development Institute for Viticulture and Enology, Valea Calugareasca, on the Potato Dextrose Agar medium, at 4°C.

In order to establish the composition of the culture medium which determine a high rate of multiplication of the fungal suspension with the preservation of the biological parameters of the strain, some different culture media were analysed.

The parameters ranging during experimentation were: carbon source (20g/l) and nitrogen source (20g/l).

A source of protein represented by the crumbled insect *Tenebrio molitor* in a concentration of 0.5 g/l was added to all the medium variants. Six different carbon sources: sucrose ($a_1$), glucose ($a_2$), maltose ($a_3$), fructose ($a_4$), lactose ($a_5$), cellulose ($a_6$) and three nitrogen sources: yeast extract ($b_1$), peptone ($b_2$) and corn extract ($b_3$) were tested.

250 ml of culture medium were distributed in sterile culture vessels and sterilised by autoclavation at 121°C and 1 atm. Each culture variant was than added with 2.5 ml of *Beauveria bassiana* fungal suspension at a concentration of $1 \times 10^6$ conidia/ml. In order to stimulate the production of the spores, the cultures were shaken at 150 rpm, for 72 hours at the room temperature, and then incubated at 25°C (as described by Jenkins et al., 1998, slightly modified).

The folowing observations and determinations were made: capacity of the vegetative multiplication, conidiogenesis, viability (germination %), tolerance to the nutrient substrate.

The capacity of the vegetative multiplication was determine by the culturing of the spores suspension on Potato Dextrose Agar medium and the incubation of the cultures at 25°C for 8 days. The measurements regarding the growth of the

fungal colony were performed periodically (after 2, 5 and 8 days). 10 randomly chosen colonies were analysed.

The microbial load was performed by using the succesive serial dilutions method. The spores were counted with Thoma chamber.

The conidia germination percentage was determined by the method developed by Inglis si col., 1993. 50 µl of fungal suspension were inoculated in 150 µl Sabouraud Dextrose Broth (SDB) liquid medium (neopeptone: 10 g/l; glucose: 20 g/l; pH at 25°C: 5, 6). The cultures were shaken for 2 hours at 120 rpm and incubated for 72 hours at 24°C. The observations were made in triplicate, after 24 hours. Conidia germination percentage was assessed by the analysis of 200 conidia for each variant.

The experimental data were statistically processed by using principal component analysis based on the XLSTAT programme.

## RESULTS AND DISCUSSIONS

In the first stage, the parameters used for the selection were the folowing: the diameter of the fungal colony after 8 days of culturing on the culture medium, the number of conidia x $10^8$ in 1 ml of fungal suspension and the germination of conidia after 24 and 72 hours (Table 1).

*Table 1*

**Analytical table**

| Variant | Diameter of fungal colony (cm) | Number of conidia x $10^8$/ 1 ml | Conidia germination (%) | |
|---|---|---|---|---|
| | | | 24 hours | 72 hours |
| a1b1 | 2,93 | 6.93 | 64 | 74 |
| a1b2 | 2,70 | 7.04 | 61 | 72 |
| a1b3 | 1,90 | 4.90 | 66 | 76 |
| a2b1 | 2,48 | 3.89 | 50 | 62 |
| a2b2 | 2,24 | 4.01 | 61 | 72 |
| a2b3 | 1,82 | 1.87 | 58 | 69 |
| a3b1 | 2,22 | 3.83 | 60 | 72 |
| a3b2 | 1,88 | 5.17 | 68 | 80 |
| a3b3 | 1,54 | 3.23 | 61 | 72 |
| a4b1 | 2,40 | 4.46 | 33 | 45 |
| a4b2 | 2,34 | 3.73 | 61 | 73 |
| a4b3 | 1,44 | 2.80 | 58 | 71 |
| a5b1 | 3,50 | 9.44 | 80 | 93 |
| a5b2 | 3,68 | 9.06 | 83 | 94 |
| a5b3 | 2,80 | 8.03 | 81 | 92 |
| a6b1 | 1,72 | 1.76 | 28 | 50 |
| a6b2 | 1,50 | 1.77 | 30 | 42 |
| a6b3 | 1.24 | 1.30 | 63 | 44 |

The first specific information for the factorial analysis was provided by the total explained variance, which is presented in table 2. The main components formed as a linear combination between the variables were characterized by a value and a variance. The variance explained by each factor

was distributed as follows: the first extracted component was equal to 2.570 and was the linear combination which took the maximum possible from the initial data, 85.66% respectively. The second principal component took much less of the variance (12.04%), was equal to 0.361 and the third variant took 2.3% from the variance of the data heaving the value of 0.069. The first two extracted components acquired 97.7% of the total variance of data. The reduction dimensionality of the data was achieved (Table 2).

*Table 2*

**The explanation of the variance**

| Value | PC 1 | PC 2 | PC 3 |
|---|---|---|---|
| Eigenvalues | 2,570 | 0,361 | 0,069 |
| Variability (%) | 85,664 | 12,039 | 2,296 |
| Cumulative (%) | 85,664 | 97,704 | 100,000 |

The first principal component was positively correlated with all three variables. The second component was positively correlated only with the variable "Conidia germination after 72 hours (%)", (0.484) and negatively with the other two, and the third component was positively correlated only with the variable "number of conidia x $10^8$", (0.203). Since the variables are correlated with at least one of the main components, the variables can be considered responsible for the variance of the data (Table 3).

*Table 3*

**The correlations between the initial variables and the principal components**

| Variabiles | PC 1 | PC 2 | PC 3 |
|---|---|---|---|
| Diameter of the fungal colony | 0,928 | -0,338 | -0,155 |
| Number of conidia x $10^8$ | 0,973 | -0,111 | 0,203 |
| Conidia germination after 72 hours (%) | 0,873 | 0,484 | -0,062 |

For each variable was given a score based on the projection of the variables on the two main axes. The score provided information concerning the coefficients of each variable involved in the component description.

The score obtained for each relation was graphically represented in figure 1.

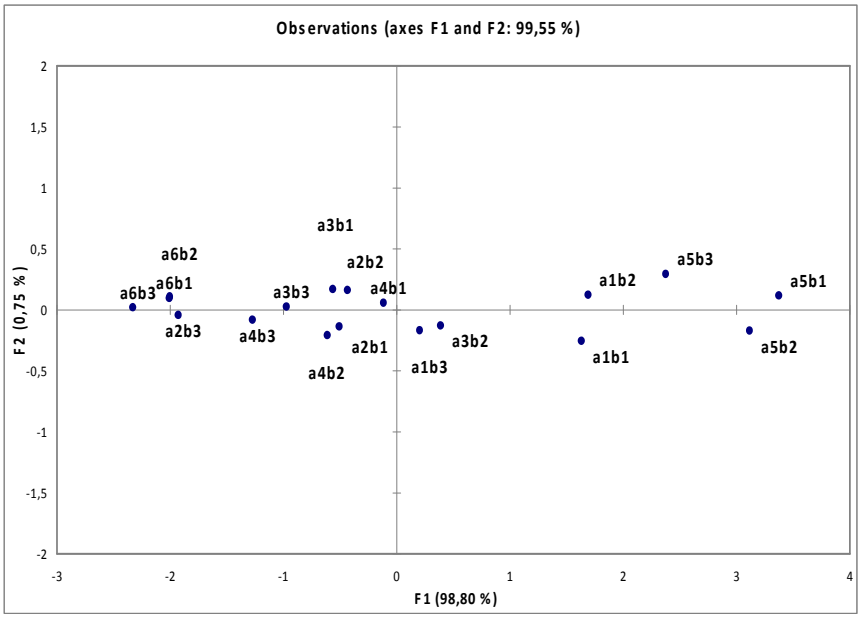


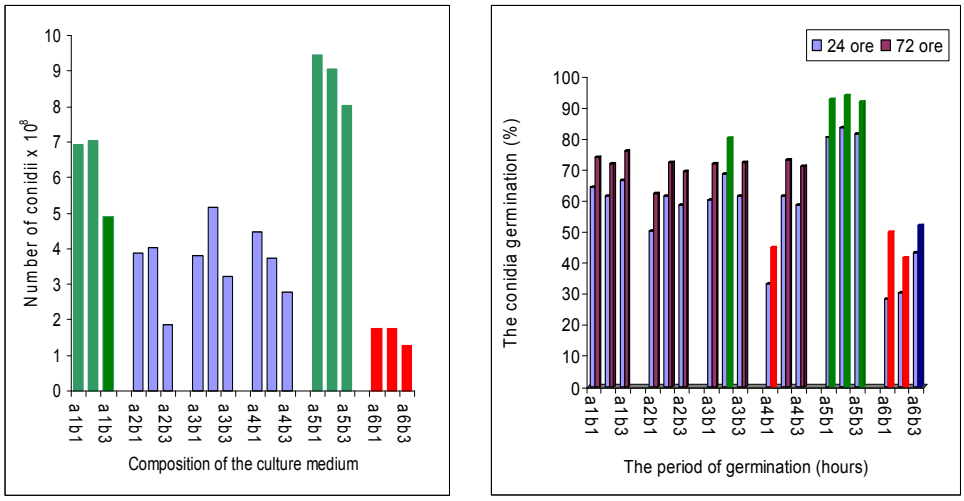

**Fig. 1** - Graphically representation of the PCA1 and PCA2 points

Statistical, the experimental variants were grouped as follows: the variants $a_5b_1$ and $a_5b_2$ were clearly different from the other variants, in a positive way, for all the variables taken in the study; the variants $a_6b_3$, $a_6b_1$ and $a_6b_2$ presented the lowest values for the studied variables; the other variants showed lower values and uneven in comparison with the first group; among the six carbon sources tested, lactose ($a_6$) was the best carbon source for the multiplication and sporulation of the fungus; the combinations of lactose with yeast extract and peptone resulted in a higher production of spores; the use of cellulose as a carbon source in combination with all three sources of nitrogen conducted to the worst results, both in terms of vegetative growth and the amount of spores produced.

The largest amount of conidia/1 ml was obtained on the media with lactose ($8.0 – 9.4 \times 10^8$) and sucrose ($4.9 – 7.0 \times 10^8$). The smallest amount ($1.3 – 1.8 \times 10^8$) was registered in case of variants with cellulose (Figure 2 a).

The analysis of the viability of conidia showed an average percent of germination of 60% after 24 hours and 70% after 72 hours. Maximum values were obtained for the combination lactose and yeast extract and minimum values for cellulose and yeast extract (Figure 2b).

Folowing the obtaining results, the best variant of the medium for the multiplication of the fungal suspension was selected. This was $a_5b_2$ variant with the following composition: lactose 20g/l, peptone 20g/l, and the crumbled insect *Tenebrio molitor* 0.5g/l.




a) Number of conidia b) Germination of conidia

**Fig. 2** - The conidiogenesis depending on the composition of the culture medium

## CONCLUSIONS

1. The nutritive substrate influenced the multiplication and the sporulation capacity of the *Beauveria bassiana* spores.

2. The best results, both in terms of vegetative growth and the amount of spores produced were obtained when the lactose was used as the carbon source. The worst results were obtained on the medium added with cellulose for all the three nitrogen sources used in the experiment.

3. The medium which was selected for the multiplication of the fungal suspension have in composition the lactose as source carbon and peptone as nitrogen source.

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