Test of Culture and Biomass Production of the Entomopathogenic Fungus *Beauveria bassiana* (Bals.-Criv.) (Vuil., 1912) on Lactoserm, Margine and Olive Pomace

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Abstract: Entomopathogenic microorganisms occupy an important place among the alternative methods of fighting against pests insect. The fungus *Beauveria bassiana* is an agent naturally present in ecosystems. It has potential to control pest populations. In the context of biological control, the present work aims to the study of linear growth of *Beauveria bassiana* on different natural environments from the food industry. They are the raw whey, water and pomace, and followed the development of the fungus through a trial production of biomass on deproteinized whey.

Key words: *Beauveria bassiana*, entomopathogenic, biomass production, whey, vegetable, olive pomace.

1 Introduction

Biological control against insect pests is the use of living organisms to reduce the population of pathogens in order to reduce damage to crops, without harming other organisms, while preserving the environment.

Entomopathogenic microorganisms play an important role among alternative methods of struggle against insect pests. The effectiveness of the fungus *Beauveria bassiana* made him the best bio-insecticide [1]. For the extensive use of bio-insecticide, a production of an important biomass is required by simple techniques and inexpensive. So, using agro-industrial residues, knowing that the agricultural and food industries generate significant amounts of by-products, which are most little or no recovery, the discharge in the wild is a threat to the environment. However, they can be exploited for biotechnological purposes.

2. Materials and Methods

2.1 Biological Material

In this study, one single isolate of the fungus *Beauveria bassiana*, isolated from soil in the region of Chréa and identified in the laboratory of Microbial Biotechnology, Faculty of Sciences, University of M'hamed Bougara Boumerdes was used.

2.2 Methodology

2.2.1 Study of Mycelial Growth of the Fungus *Beauveria bassiana*

To analyze the rate of growth of *Beauveria bassiana*, three different liquid substrates were used to cultivate the fungus which are the settings: PDA, whey and vegetable.

2.2.1.1 Procedure

- The agar-based culture media natural was prepared and then pours into Petri dishes;
- Cut with a cookie cutter explants from a fungal culture on OGA environment, then place them in a way reversed on the new agar culture;

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- Incubate for 5 days at a temperature of 25 °C;
- Cut a 2nd time with a punch of a 1.1 cm diameter circles already inoculated explants, then place in the center of the box containing the same culture environment;

- Incubate for 15 days at a temperature of 25 °C.

2.2.1.2 Expression of Results

Mycelial development is measured every three days in each box with a ruler until growth reached the edges of the Petri dish. Linear growth is given by the Eq. (1) [2].

$$L = D - d/2 \quad (1)$$

L: mycelia growth;

D: colony diameter of fungal colony.

The means of mycelium growth were calculated as Eq. (2):

$$V \text{ (mm/day)} = (L_n - L_{n-1})/n \quad (2)$$

V: means of mycelium growth;

$L_n, L_{n-1}, L_{n-2}, \dots$ are the means of mycelium growth of days $n, n-1, n-2, \dots$ n : the number of days.

2.2.2 Biomass Production of the Fungus *Beauveria bassiana*

2.2.2.1 Conduct of Fermentation

For biomass production of the fungus *Beauveria bassiana*, the batch fermentation was realized in a fermenter of two liters containing 1.25 liter of deproteinized lactoserm.

- Preparation of inoculum: the preculture was carried out in two Erlen Meyer, each containing 50 mL of deproteinized lactoserm;

- Preparation of a fermenter: the fermenter and the acid-base solutions are sterilized in an autoclave for 20 minutes;

- Injection of inoculum: after checking the purity of the preculture, 40 mL of preculture at a concentration of 1.25×10^5 spore/mL was introduced aseptically in deproteinized lactoserm [3];

- Conditions of fermentation.

2.2.2.2 Following the Fermentation (Study of the Kinetics of Growth)

Samples of 25 mL each were made throughout the

three days of fermentation, to study the pH, biomass (spores/mL) and the rate of reducing sugar (g/L):

- Determination of reducing sugars is performed on the supernatants of samples centrifuged;
- Evaluation of biomass [1];
- Biomass (cell concentration) of six samples taken from the fermentation broth was evaluated by counting on a Malassez cell.

Expression of results: The cell concentration was calculated as in Eq. (3):

$$N = (n/V) \times f \quad (3)$$

N is number of cells per liter; n is number of spores counted; V is volume counting; F is dilution factor. Measuring pH uses a pH meter.

2.3 The Sporulation

By performing a test culture of *Beauveria bassiana* on the olive pomace, there was a good mycelial growth after the 15th day of incubation, therefore, it conducted a monitoring changes in the rate of sporulation this fungus on agroindustrial residue that is realized with counting the number of spores produced in each box every 72 hours for 15 days.

Procedure:

- Dry olive residue in the oven at a temperature of 104 °C;
- Mill the dried residue;
- Ass the mash through a sieve (1 mm pore);
- Moisten the powdered pomace obtained with distilled water;
- Sterilize the environment in an autoclave at 120 °C for 20 min;
- Fill the Petri dishes;
- Seed the-dishes.

Expression of results: Counting is performed on a Malassez cell, spores produced 0.1 g of olive every 72 hours for 15 days.

3. Results and Discussion

3.1 Measurement of Mycelial Growth (Linear)

The results for the measurement of mycelial growth

Table 1 The operating conditions of batch culture of *Beauveria bassiana* in the deproteinized whey.

Temperature	25 °C
pH	5
Agitation	120 trs/min
Aeration	1.30 VVM

of the strain grown on environment of *Beauveria bassiana* selected for the study of cultural preferendum are mentioned in the Fig. 1.

The best growth was observed in lactosermum followed by the PDA environment, and finally the margine. This (succession) is due to the nature of the carbon source contained in any setting. The lactosermum contain a significant content of lactose, which is a carbohydrate composed of one molecule of galactose and glucose easily degradable by *Beauveria bassiana* due to the enzyme β -galactosidase [4]. The PDA environment containing two carbon sources namely glucose and starch which makes it a favorable environment for growth of the fungus. Otherwise, the margine contains many complex sugars arabinose, cellulose and pectin, making it more difficult to degrade as compared to the other two circles [5]. The results are quite comparable with those found by various authors who demonstrated that *Beauveria*

bassiana behaves in a similar manner on organic and inorganic media [4]. Meanwhile a maximum *Beauveria bassiana* grows medium with carbon source as CaCO_3 [6].

3.2 Sporulation

The results of counting on a Malassez cell, spores produced by *Beauveria bassiana* in 0.1 g of olive every 72 hours for 15 days are recorded in the Fig. 2.

The number of spores produced by *Beauveria bassiana* on the olive pomace is growing throughout the incubation, the mycelial growth reflected by the presence of a carbon source in this environment is cellulose [5].

3.3 Conduct of Fermentation

Figs. 3-5 show the results of the parameters studied during the fermentation.

The pH of the fermentation broth decreases gradually reflecting their acidification, with some fluctuations that reveal the secretion of acid metabolites (organic acid) and basic metabolites (ammonia) by *Beauveria bassiana* [7].

The rate of sugars in the fermentation broth gradually

Fig. 1 Evolution of the linear growth in cm of *Beauveria bassiana* in margines, lactosermum and PDA.

Fig. 2 Evolution of the sporulation of *Beauveria bassiana* in olive pomace.

Fig. 3 Evolution of pH during the batch fermentation of *Beauveria bassiana* in the lactoserm.

Fig. 4 Evolution of the concentration of reducing sugars (g/L) during the Butch culture of *Beauveria bassiana* in deproteinized lactoserm.

Fig. 5 Evolution of the biomass during the batch cultures of *Beauveria bassiana* in the deproteinized lactoserm.

decreases, indicating consumption in the presence of β -galactosidase enzyme secreted by *Beauveria bassiana* [8], and tends to stabilize to the 12th and 15th days. This can be translated by the accumulation of secondary metabolites produced by the microorganism that can make the toxic environment which reduces the multiplication of the fungus and hence its metabolic activity [9]. The increase of the biomass produced during fermentation confirms the growth of *Beauveria bassiana* by the consumption of the carbon source (lactose) present in the environment.

4. Conclusion

After the biomass production test of fungus *Beauveria bassiana* on agro-industrial residues, several points were made:

The study of mycelial growth or preferendum cultural shows that the two natural environments (lactoserm and margine) are suitable for the development of the strain of *Beauveria bassiana*, however, the best growth was recorded in the lactoserm;

The result of the rate of sporulation of *Beauveria bassiana* on the olive pomace shows that this by-product of the agricultural industry is suitable for the growth of fungus and can be used as an

environment;

Through the results found, the fungus *Beauveria bassiana* happens to grow easily on the natural environment with low nutritional value, which let to assume that a species is not demanding on the composition of culture environment.

It is much more interesting to project the test widely to make it happen in biotechnology units working in the service of agriculture and the environment, setting up experimental devices that provide the greatest biomass yield a much most important and which when converted into bio-insecticide can contribute to biological control.

Furthermore, the use of releases of agricultural and food industries, for biotechnological purposes, such as culture environment in the case of these studies help to reduce the environmental pollution.

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