BIOLOGICAL INSECTICIDE INOCULUM FOR PLANT PROTECTION AGAINST ROOT PESTS

INSECTICIDE BIOLOGICE INOCULANTE DESTINATE PROTECȚIEI PLANTELOR FAȚĂ DE ATACUL DĂUNĂTORILOR DE RĂDĂCINĂ

FĂTU Ana-Cristina[1], CIORNEI C[2]., FĂTU V.[1], LUPĂŞTEAN Daniela[3], PAVEL Otilia[4], ANDREI Ana-Maria[1]

Abstract. *Entomopathogenic microorganisms isolated from natural outbreaks were tested both in laboratory and field conditions in order to evaluate the posibility of their usage as inoculative bioinsecticides for root pest control. The laboratory results lead to a selection of some autochtounous Beauveria brongniartii strains with bio-ecological potential, adapted to romanian pedoclimatic conditions and identified as the principal source of biological material for the bioinsecticides production. Field tests were conducted in moldavian forest nurseries located in different regions and infested with Melolontha melolontha. In this paper we present the results concerning the method of entomopathogenic bioinsecticides application and their biological efficacity in European cockchafer control.*
Key words: *Beauveria brongniartii, Melolontha melolontha,* bioinsecticides

Rezumat. *Microorganismele entomopatogene izolate din focare epizootice naturale au fost testate în condiţii de laborator şi câmp, în vederea evaluării posibilităţii de utilizare a acestora sub formă de biopreparate inoculante destinate combaterii unor dăunători de rădăcină. Rezultatele experimentelor desfăşurate în condiţii de laborator vizează selecţia unor tulpini autohtone de Beauveria brongniartii, cu potenţial bio-ecologic adaptat condiţiilor pedo-climatice din România, identificate ca sursă de material biologic pentru obţinerea de biopreparate. Testele de câmp s-au desfăşurat în pepiniere silvice din judeţul Suceava, situate în diferite condiţii staţionale şi infestate cu Melolontha melolontha. În lucrare sunt prezentate rezultate privind metoda de aplicare a biopreparatelor entomopatogene şi eficacitatea biologică a acestora în combaterea cărăbuşului de mai.*
Cuvinte cheie: *Beauveria brongniartii, Melolontha melolontha,* bioinsecticide

INTRODUCTION

Among root pests, beetle species belonging to Scarabeidae familiy are now the first biotic stress factors in forest nurseries. In the last two decades *Melolontha melolontha* L. (May cockhafer) recorded mass propagation and produced significant damage in Romania.

The limited use of chemical insecticides to control these pest categories imposed a special focus on integrated control measures and among these, on

[1] Research-Development Institute for Plant Protection, Bucharest, Romania
[2] Forestry Research Station, Bacău, Romania
[3] „Ştefan cel Mare" University, Suceava, Romania
[4] Museum of Natural Sciences, Bacău, Romania

biological control using entomopathogenic microorganisms (Ferron, 1978). The most effective biopesticides used in Europe for *M. melolontha* control are based on entomopathogenic fungi, *Beauveria brongniartii* (Sacc.) Petch. being recognized in this respect (Ciornei et al., 2010).

MATERIAL AND METHOD

Obtaining the biological insecticides

Beauveria brongniartii "wild" strains were isolated from *Melolontha melolontha* larvae covered by fungal mycelium, in natural epizootic outbreaks (tab. 1).

Selection of the biological material source for bioinsecticide production was assessed by measuring the biological potential of each monosporal isolate through a method involving the estimation of specific biological parameters, including germination percentage of spores, sporulation on insects, insect mortality rate and mortality distribution, life cycle of the isolated organism.

Table 1

***B. brongniartii* biotypes tested for fungal strains selection**

Strain	Date of isolation	Place of isolation	Source of isolation
Bg01	18.05.2007	Roman nursery (Neamț county)	*M.melolontha* (L_2)
Bg02	25.05.2007	Tg. Neamț - Dumbra nursery (Neamț county)	*M.melolontha* (L_3)
Bg03	26.06.2008	Gura Humorului nursery (Suceava county)	soil

In order to obtain fungal biomass, multiplication of the monosporale fungal isolates was made in two steps:

(1) Vegetative mycelium (fungal inoculum liquid) was obtained from microbiologically pure colonies, inoculated on solid artificial medium, incubated at 25^0C, until complete sporulation and then transferred into liquid culture medium based on glucose, corn extract and salts, that was distributed in fermentation flasks and incubated under agitated conditions, 24 hours at 27^0C.

(2) Sporulated mycelium (solid fungal inoculum) was obtained by inoculation of a natural solid substrate (barley seeds) with vegetative mycelium (liquid fungal inoculum). To do so: barley seeds were weighed, washed under running water and distributed in autoclavable plastic bags; after sterilization (121^0C, 30 min.), the substrate was inoculated with 1.6% fungal inoculum liquid obtained in the previous stage; inoculated natural substrate was then incubated under stationary conditions at 25^0 C for 25 days.

Biological insecticides testing under field conditions. Pest infestation of root forest nurseries in Moldova was established by performing soil surveys. Entomopathogenic bioformulations were applied in different variants (doses), in nurseries located in different site conditions.

Experimental devices were latin rectangle type, including the control area. Treatment was made by manual spreading on soil surface. The soil preparation before the biological treatment as well as the incorporation of the biological insecticides into the soil was made with a motor hoe, when the treatment was done between rows of seedlings, ie disk cutter or drill towed by tractor, when treatments were applied over the entire experimental field. The biological efficacy of the treatment was assessed by comparing the number of dead larvae infected by *B. brongniartii* to the total number of living larvae initially present in the soil.

RESULTS AND DISCUSSIONS

A *B. brongniartii* strain isolated in Roman nursery, Neamt county, from a second instar *M. melolontha* larva (fig. 1) was selected for multiplication in laboratory conditions in order to obtain biological insecticide (Andrei, 2004).

Fig.1 - Mycosed *M.melolontha* larva, from wich the *B. brongniartii* (Bbg) strain was isolated

After 6 days incubation at 25°C, on artificial solid medium, Bbg was presented in the form of lanose colonies, velvety to powdery, at first white, then yellow; on potato-glucose-agar (PDA) medium, reverse colony was white, and a red pigment was diffused in the medium; exudate absent. Microscopically, they revealed hyphae hyaline, septate, with regular contour, conidiogenous basal cells, unicellular predominantly ellipsoidal conidia, hydrophobic, small (approx. 4 μm) with a zigzag-shaped rachis.

Production and formulation technology of Bbg strain to obtain biological insecticide included the following steps:

(I) Obtaining a "stock culture" of *B. brongniartii* (Bbg). Bbg monosporale strain from original isolate, kept in glass tubes at 4^0C on standard mycological medium (PDA) and being periodically transferred on fresh medium was analyzed in terms of biological key parameters. The following values were obtained: 100% microbiological purity, 92% sporulation after 72 hours, 156 g / l biomass, 89% *Tenebrio molitor* (Andrei et al., 2001) test insect mortality (biological titer of the fungal suspension was 12.5 x10^9 conidia / ml).

(II) Production of „inoculum units" took place in two stages: (II.1) obtaining of laboratory inoculum by multiplication of biological material from the stock culture on agarised medium, seeding a total of 30 tubes/strain, followed by incubation (72 hours, 25^0 C); (II.2) obtaining the batch inoculum by inoculation of laboratory inoculum on liquid medium, under agitation conditions (48 hours, 27^0 C).

(III) Obtaining of biologically active fungal biomass by pouring the liquid fungal inoculum on solid nutrient substrate (barley grains) to obtain conidia, the fungal bioactive substance.

(IV) At the end of incubation period, when the nutrient substrate fermentation was complete, the bioinsecticidequality control was performed by measuring virulence on test insects.

The results of biological characterization of experimental bioformulation batches based on *B.brongniartii* obtained at the RDIPP is indicated in Table 2; the results shown that the average conidia production per gram of final product was 1.55 x 10^{10}, the percentage of conidia viability ranged between 88-99%; the results of the pathogenicity test on insects showed that an 86% average percentage of larval mortality corresponded to 98.5% average percentage of mycosis induced by the "active substance "of fungal bioformulation.

Table 2

Biological characterization of experimental bioformulation batches (*B. brongniartii*) obtained in the year 2010

Batch number/ Data	Conidia viability (%)	Conidia number x 10^{10}/g	(%) Insect mortality / mycosis	Batch number/ Data	Conidia viability (%)	Conidia number x 10^{10}/g	(%) Insect mortality / mycosis
1/9.01	97	1,6	89/100	9/16.02	92	1,7	92/100
2/16.01	99	1,8	92/100	10/22.02	97	1,5	86/100
3/18.01	96	1,9	83/95	11/27.02	88	1,2	93/100
4/22.01	93	1,5	88/90	12/2.03	93	1,8	74/100
5/27.01	95	1,5	94/100	13/11.03	90	1,6	79/100
6/30.01	97	1,2	73/100	14/24.04	97	1,6	91/97
7/2.02	95	1,4	90/100	15/2.05	96	1,7	93/100
8/7.02	98	1,7	87/95	16/13.05	94	1,2	89/100

Experimental biological control in nurseries located in the hill mountain area. In the course of the year 2011 the treatments were made to control may cockhafer by using biological insecticide based on *B.brongniartii*. Treatments were applied in nurseries located in different site conditions, using different doses: V_1 = 100 kg / ha, V_2 = 150 kg / ha and V_3 = 200 kg / ha.

In Cerbărie nursery (Fig. 2), Malini forest district (Suceava), biological insecticide was tested in an experimental device (500 sqm x 3 variants and a control surface of 160 sqm).

Fig. 2 – Experimental device in Cerbarie nursery (altitude 420 m)

Bioinsecticide (fig. 3) was applied at 20.04.2011.

Fig. 3 - Bioinsecticide weighed for application

First observations showed that the *M. melolontha* larvae infestation was strong (1.0 L_3/m^2 in V_2 and control) and very strong (1.7 L_3/m^2 in V_1 and 1.3 L_3/m^2 in V_3). Treatment efficacy results can be found in fig. 5.

In Tărnicioara nursery from Suceava district (700 m altitude), the treatment was conducted on 30.04.2011. Each variant consisted of 350 sqm and the control surface measured 100 sqm for. At the time of the first observations of efficacy (05.30.2011), 30 days respectively after the biological treatment, *M. melolontha* infestation was very strong in all variants: 1.7 L_3/m^2 in V_1 and control, 1.3 L_3/m^2 in V_2 and 2.0 L_3/m^2 in V_3. Mortality caused by biological treatment was considered when dead larvae were covered by the fungal mycelium (fig. 4).

Fig. 4 – *M. melolontha* larvae covered by the fungal mycelium after the treatment

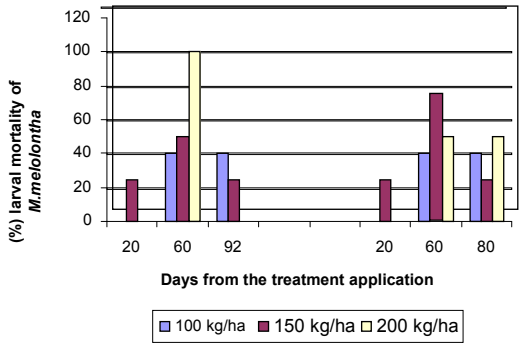

Fig. 5 – Treatment efficacy at different time
(20-92 days, in Cerbarie nursery, 20-80 days in Tărnicioara nursery respectively)

CONCLUSIONS

1. Surveys on the *B. brongniartii* occurence revealed natural epizootic outbreaks in northern Romanian forest nurseries.

2. A technology solution of the process for obtaining fungal biomass with the increase of *B. brongniartii* active substance has been streamlined, on the one hand by using as source of biological material for the production of biological insecticides local strains of *B. brongniartii*, with a high bio-ecological, biotechnological and pathological potential and on the other hand, by exploiting the microcyclic character of fungal strains sporogenesis, in order to obtain high yields of spores (conidia) in a short time.

3. The biological treatment efficacy occurred in all experimental variants. There was a positive correlation between application dose and effect. Dose of 100 kg / ha registered 80% effectiveness. Doses of 150 kg / ha and 200 kg / ha were 100% effective, causing mortality to the third instar larvae. Maximum effectiveness occurred after 60 days, in both experimental fields.

REFERENCES

1. **Andrei A.M., Bratu E., Tica D., 2001** - *Pathological characterization of some Beauveria bassiana (Bals.) Vuill. strains through the biotest procedure.* Analele Institutului de Cercetari pentru Protectia Plantelor. v. 31. p.141-146.
2. **Andrei A.M., 2004 -** *Elemente de tehnologie pentru obtinerea unor insecticide biologice pe baza de Beauveria bassiana.* Lucrarile celei de a 6-a Conferinte nationale pentru protectia mediului prin mijloace biologice si biotehnice. Ed. Pelecanus Brasov, p. 8–13.
3. **Ciornei C., Andrei A.M., Arinton M., Lupastean D., Apostol B., Popa N., Cardas G., Rotariu C., Cucos V., 2010** - *Use of fungal insecticides with B. brongniartii for biological control of may cockhafer (Melolontha melolontha* L.) *in forest nurseries in Romania.* IUFRO Working Party 7.03.10 - methodology of forest insect and disease survey in central Europe. Workshop on "Biotic Risks and Climate Change in Forests", 20-23 sept. 2010, Freiburg, Germania.
4. **Ferron P., 1978 -** *Biological Control of Insect Pests by Entomogenous Fungi.* Annual Review of Entomology. v. 23, p. 409-442.