ABSTRACT

« STUDY ON THE FACTORS DETERMINING THE EFFICACY OF THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BRONGNIARTII* IN THE CONTROL OF EDAPHIC PESTS »

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Keywords: *Beauveria brongniartii*, *Melolontha melolontha*, entomopathogen, bioinsecticide, nursery

In Romania, scarabs (Coleoptera: Scarabeidae) are considered to be very important pests for agricultural, horticultural and forestry crops. Their larvae live in the soil, and the adults on tree crowns. The larvae attack the plant's roots, causing substantial losses in quality and quantity. In nurseries and forestry plantations, the root pests are the main biotic factor of stress. In the last two decades, *Melolontha melolontha* L., but also other species of scarabs (*Polyphylla fullo, Anoxia pilosa, A. orientalis, Anomala dubia, A. solida, Amphimallon solstitiale, A. caucasicum, Rhizotrogus aestivus, R. aequinoctialis, Anisoplia sp.*) recorded mass multiplication and caused significant damage in crops in forestry nurseries. Among scarabs, the most common species that can cause damage to the seedlings in forest nursery is the May beetle (*M. melolontha)* that during 2012-2013 was in eruption phase in most areas of the country. Larvae (white worms) cause the biggest damage during the last larval stage before pupation. Limiting the use of chemical insecticides for the control of this pest category required special attention to alternative control measures. Of these, the use of the entomopathogenic *Beauveria brongniartii* (Sacc.) Petch (Hypocreales: Clavicipitaceae) is of real interest, proving to be the most effective biologic control agent for the May beetle.

The doctoral thesis entitled "Study on the factors determining the effectiveness of the entomopathogenic fungus *Beauveria brongniartii* in the control of edaphic pests" presents a series of researches that follow the stages of the development of a bioinsecticide.

The first stage consisted in collecting and isolating fungal pathogens from natural outbreaks of *M. melolontha* larvae, gathered from forest nurseries with strong infestations. Morphological identification of fungal isolates was followed by their genetic characterization based on separate molecular markers (ITS, EF-1α, 18s and 28s). A strain of *B. brongniartii* from an international

collection of microorganisms was also used. Phylogenetic sequencing and reconstruction analyses were carried out comparing with other existing strains in the database. The results have demonstrated a high degree of kinship within Romanian isolates at the infraspecific level, forming a compact subclade within the *B. brongniartii* clade.

The effect of temperature and relative humidity on *B. brongniartii* conidia germination was studied, the most important factors for initiating the process of infection, was studied. Germination was investigated at temperatures between 4 ° and 33 °C on solid culture medium. The effect of relative humidity was tested using saturated solutions. The results showed that germination occurred at temperatures between 15.5 and 25 °C. None of the three fungal strains tested (ICDPP # 2, ICDPP # 3 and ICDPP # 4) geminated at low temperatures (4 ° and 10 °C). A single strain (ICDPP # 3) germinated at high temperatures (30 ° and 33 °C). Incubation of conidia at a low relative humidity level (29% UR) inhibited the germination of the strains. The results obtained showed that the relative humidity ranging from 52.6 to 100% is not a limiting factor for conidial germination.

In the next step, the three strains of *B. brongniartii* were tested on *M. melolontha*, *A. villosa* and *A. solstitiale* larvae in order to select a virulent strain to be used in further investigations. Evaluation of pathogenicity of *B. brongniartii* was done in parallel with a native isolate of *Metarhizium anisopliae* (Metsch.) Sorokin. Third instar larvae were treated by immersion in conidial suspensions with a titer of $1x10^7$ conidia / ml. The results showed that the highest mortality rate was due to infection with *B.brongniartii* (100% for *M. melolontha* larvae and 60% for *A. villosa* larvae) after 60 days of incubation. *M. anisopliae* caused a low mortality rate (below 35%) for all the insect species. The strains of *B. brongniartii* showed the same degree of virulence against *M. melolontha* larvae.

The biotechnological processing stage of the *B. brongniartii* strain used to obtain granular bioinsecticide aimed at selecting a culture medium suitable for an adequate quantitative and qualitative fungal inoculum. The influence of the composition of four liquid culture media (Catroux, Paris, Goral and Kondryatiev) on the production of propagules, myelian biomass, and the shape and size of fungal propagules of the ICDPP # 2 strain, was evaluated. In Catroux, the highest concentration of propagules ($5.7 \pm 4.4 \times 10^9$ propagules / ml) was obtained after 96 hours, and the highest amount of micelian biomass (9.41 ± 3.53 mg / ml) was obtained after 120 incubation hours under shaking conditions (150 rpm) at 25 ° C. Whether or not the pathogenicity of the bioinsecticide is influenced by the composition of the culture medium in which propagules subsequently used as inoculum for the final product have been formed, was also verified. For this purpose, *M. melolontha* larvae were treated with conidial suspensions (1×10^7 conidia / ml) obtained from the bioinsecticide.

The evaluation of larval mortality over 52 days showed that there are no statistically significant differences in average survival time (log-rank 0.39); the shortest survival time (27 days) was recorded following treatment with bioinsecticide inoculated with the fungal culture obtained on Catroux medium.

Based on the biotechnological and pathogenic properties, the ICDPP # 2 strain was selected for field application in the form of a fungal bioinsecticide formulated as barley kernels colonized by fungal sporulated mycelium (BioMelCon) for control the *M. melolontha* larvae in the soil, or formulated as a conidial suspension for foliar application against adults of *M. melolontha*, in nurseries and forest plantations.

The field tests were carried out during 2010-2013, in nurseries from Botosani, Neamt and Suceava counties, located in different stational conditions, showing a very high level of *M. melolontha* infestation (> $1L_3$ / m.p.). The treatments were applied for two consecutive years at doses ranging from 100 to 200kg / ha, the treated areas accumulating concentrations of 2 to 5 x 10^{15} conidia / ha. Treatments against *M. melolontha* larvae have reduced population density by 66,7-100 %, mortality level having a tendency to increase with the increasing dose of the bioinsecticide administered into the soil. The results concerning the effect of treatments applied to the soil for two consecutive years have not consistently demonstrated a positive correlation between the bioinsecticide dose and the larval mortality induced by it. Thus, at the cumulative dose in the two years, of 200 kg / ha the mortality varied from 43,8% to 100%, at a dose of 300 kg / ha from 50 to 69,7% and at a dose of 400 kg / ha mortality was between 75% and 100%. The results of the observations made in the experimental fields regarding the density of the *M. melolontha* populations and the degree of fungal colonization of the soil before and after applying the treatment confirm that reduction of the May beetle populations was due to treatments with *B. brongniartii*.

The presence of the May beetle flight in the spring of 2012 in some areas in northern Moldova allowed for testing the some methods against on adults: by treating the food represented by the host plants, as well as by the direct application of *B. brongniartii* conidia on adults caught in light traps; the procedure of bathing the adults of *M. melolontha* followed by their release into the field was applied. The purpose of this study was to evaluate the possibility of using the adult as a vector of entomopathogen in breeding areas. The effect of direct application of bioinsecticide on adults was manifested after one year with an efficacy of 100%. In the case of foliar treatments in nurseries infected with adults of *M melolontha*, in the autumn of the same year, a 100% efficacy was registered by measuring the larval density in the soil.

The effect of treatment with *B. brongniartii* on edaphic mesofauna within two years was also evaluated. The results of the quantitative analysis of edaphic fauna showed that the density of edaphic microarthropods was about 2-fold lower at one year after treatment, compared with the control, in the nurseries tested; after two years, there was an obvious recovery of the population. From a qualitative point of view, both the control samples and those from the perimeters under treatment, the numerical dominance of the mites against the insects were observed, ranging from 61,7-99,4% of the total mesofauna. Insects were represented mainly by colemboles (over 70%), which, however, lacked in the variants analysed one year after application of the treatments, while the rest had very low densities, in the whole mesofauna, with low percentage values, only 0,78% - 3,73%. The absence of colemboles in treatment variants applied in 2012 showed that they are one of the affected groups, the effect being maximum one year after biological treatment, as in the case of other groups of microarthropodes, eg oribatides. It was also found that in the second year after applying the treatment the structural parameters of oribatide cenoses had a tendency to return to comparable values to the control, so the impact of the treatments was not a lasting severe one.

The results of the analysis of the main indicators of quality and fertility of the soil resources on which experiments were located revealed that bioinsecticide treatments led to a slight increase in humus content and baseline saturation compared to the control. An increase in microflora metabolism has also been noticed. Bioinsecticide based on *B. brongniartii* had a positive effect on the soil respiration, dehydrogenase activity and on the biological synthetic indicator in the tested nurseries. The most relevant positive effects were observed in the period immediately after applying the bioinsecticide.