INSECTICIDAL POTENTIAL ASSESSMENT OF ENTOMOPATHOGENIC MICROORGANISMS AGAINST STORED PRODUCT INSECTS

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Abstract: In this paper are presented the results of laboratory tests aimed to evaluate the virulence of some entomopathogenic fungi isolated from natural epizootic outbreaks, against specific stored products pests. Fungal material in doses ranging from 2 to 4 x 10^3 *B. bassiana* conidia/g was applied on the following growth media: (i) synthetic media Hydak, for *Plodia interpunctella* (Hubner), *Galleria mellonella* (L.), *Oryzaephilus surinamensis* (L.), *Tenebrio molitor* (L.), (ii) grains, for *Sitophilus granarius* (L.), (iii) mix flour with cornmeal-milk powder, for *Tribolium castaneum* (Herbst). Statistical analysis of the results was based on the mortality rates recorded at different periods and consisted in the quantification of biological activity (LD-50). Test insects, excepting *T. castaneum* beetle adult stage, showed high susceptibility to *Beauveria bassiana*. The results demonstrated the possibility to use the entopathogenic fungi belonging to the R.D.I.P.P. useful microorganisms collection in biological control of stored products insects.

Key words: *Beauveria bassiana, Tribolium castaneum, Sitophilus granarius, Oryzaephilus surinamensis, Tribolium castaneum, Tenebrio molitor, Plodia interpunctella, Galleria mellonella*

INTRODUCTION

Beauveria bassiana (Bals.) Vuill. is an enthomopathogenic fungus that grows naturally in a wide range of habitats (Steinhaus, 1956). It is a facultative pathogen infecting species from most insect orders, being an important agent for controlling pests (Andrei, 1998).

Protection of stored products with non-toxic methods was an important concern in recent years at the R.D.I.P.P. Bucharest (Ciobanu and Drosu**,** 2009, Drosu et al., 2011).

In this paper are presented the results of the studies that have been assessing the effectiveness of Romanian experimental bioinsecticides based on entomopathogenic fungi *B. bassiana* against stored product pests. The *B. bassiana* experimental bioinsecticides used in this experiment are already known as having good efficacy in agricultural crops and forests protection.

MATERIAL AND METHOD

It was used Romanian *B. bassiana* strains isolated from natural epizootic outbreaks, to evaluate the susceptibility of stored product pests to fungal infection. There are used the following insects: grain weevil, adults (*Sitophilus granarius*), sawtoothed grain beetle, adults (*Oryzaephilus surinamensis*), the red flour beetle, larvae (*Tribolium castaneum*), flour beetle, larvae (*Tenebrio molitor*), dried fruit moth, larvae (*Plodia interpunctella*), greater wax moth, larvae (*Galleria mellonella*). Some of them are presented in fig. 1-3.

Fig. 1 *Sitophilus granarius*

Fig. 2 *Oryzaephilus surinamensis*

Fig. 3 *Tribolium castaneum*

Insects were reared under controlled conditions, on natural growth media with specific nutrients, at RDIPP. Adults and larvae (L_2-L_3) were used in biotests. After the biological treatment application, dead larvae and adults on treated growth media were collected and incubated in wet rooms. In fig. 4-6 are presented some of test insects infected by *B. bassiana*, after treatment application.

Fig. 4 *Plodia interpunctella*

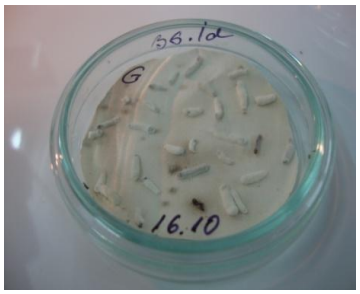


Fig. 5 *Galleria mellonella* larvae

Fig. 6 *Tenebrio molitor* larvae

Insects were inoculated by contact with *B. bassiana* fungal bioproducts. There were used two *B. bassiana* strains belonging to the R.D.I.P.P. useful microorganisms collection, respectively BbSc strain (host insect *Sciara* sp.) and BbId strain (host insect *Ips duplicatus*). *B. bassiana* strains were preserved on agar culture medium, in sporulated form, at 4ºC and periodically refreshed by successive passages on PDA medium.

To obtain the fungal bioproduct the following steps were made: 0, 500 g barley (*Hordeum vulgare*) mixed with 500 ml distilled water was placed in autoclavable bags and sterilized (121ºC, 60 min). After 24 hours, the autoclaved barley was inoculated with BbSc and BbId conidial suspensions. After 14 days, the fungus has developed and covered the nutrient medium. (Fig. 7).

Fig. 7 Fungal bioproduct (*B. bassiana* on *H. vulgare* grains)

Insects were inoculated by contact, left for 30 min. in Petri dishes with the fungal bioproduct and then transferred in vials with perforated lid. (Fig. 8-10).

Fig. 8 *Plodia interpunctella* larvae on fungal bioinsecticid

Fig. 9 Transfer of larvae

Fig. 10 *Tenebrio molitor* larvae on Hydak medium

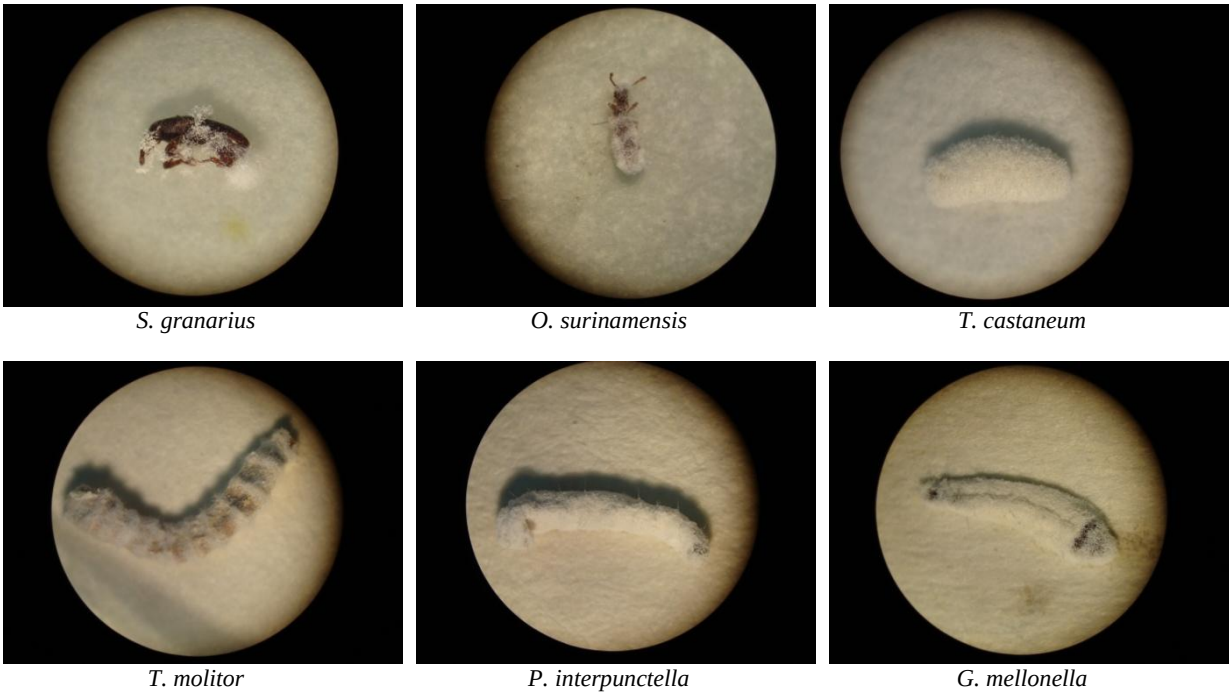
It was also tested a *B. bassiana* bioproduct formulated as conidial suspension, to be directly applied on *P.interpunctella* larvae (L_3, L_4).

By successive dilution of the aqueous suspension, resulted a bioproduct with a biological titre of 2-4x10^3 conidia/ml.

After the larvae died, they were transferred in wet rooms, incubated at 25^0C and 50-90% relative humidity and the specific symptoms of *B. bassiana* mycosis were analised.

RESULTS AND DISCUSSION

To determine the mortality percentage of insects tested were taken into account only the larvae and/or adults showing mycosis symptoms in 48-72 hours after the biological treatment (Fig. 11).



S. granarius *O. surinamensis* *T. castaneum*

T. molitor *P. interpunctella* *G. mellonella*

Fig. 11. *Beauveria bassiana* on stored products insects tested in the experiment

After evaluating individuals treated by inoculation contact method, it has been found that: (i) *S. granarius* recorded 20% mortality after the treatment with 10% *B. bassiana* bioproducts (BbSc and BbId); (ii) *O. surinamensis* recorded 20% mortality after the treatment with BbId bioinsecticide, respectively 3.3% mortality after the treatment with BbSc bioinsecticide; (iii) *T. castaneum* recorded 36.67% mortality after treatment with BbId bioinsecticide; (iv) *T. molitor* showed 3.3% mortality after

BbSc and BbId treatment; (v) *P. interpunctella* recorded 80% mortality after BbSc and BbId treatment; (vi) *G. mellonella* registered 70% mortality after BbSc and BbId treatment. The results are presented in fig. 12.

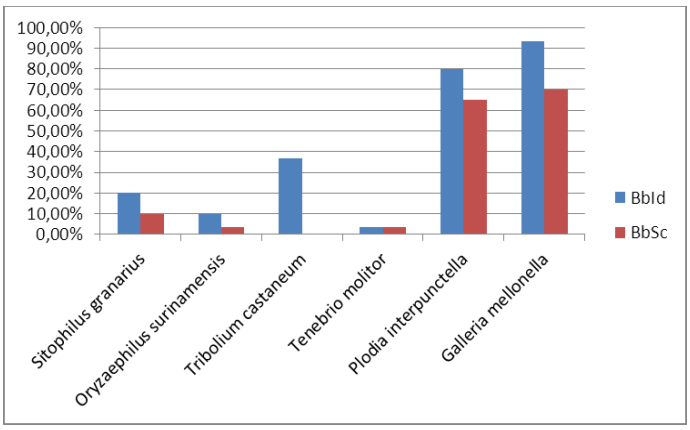


Fig. 12. *B. bassiana* bioproducts originated from two different fungal strains, tested on stored products insects

The experiment demonstrated that high doses of conidia and repeated manipulations kills larvae by other means than infectivity (trauma due to massive cuticular breaks etc) and can not be processed statistically. Older larvae (L_4) have proven resistant to manipulation, but fungal contamination was prevented because the pupae emergence; the mortality percentage had similar values (6-8%) with the control and were not taken into account in the statistical analysis.

Relative humidity between 41-94% did not affect the interaction between *B. bassiana* and test insects. The results showed that the insect cuticle surface (especially in the larval stage) provides a favorable microclimate for *B. bassiana* conidia germination. It was also found that a temperature of 25°C stimulates the fungal contamination; at this temperature, the larval mortality starting after 48 hours of contact with the pathogen.

CONCLUSIONS

The *B. bassiana* isolated in Romania, from natural epizootic outbreaks and tested in laboratory conditions have expressed different virulence against stored product insects. For insects, the most important causes of variation in susceptibility are extrinsic factors (temperature, humidity, type of food, population density, etc.) and intrinsic factors (larval age, physiological condition etc.). Thus, the immature stages are important for anatomical reorganizations and changes in metabolism (for eggs, pupae), growth and changes associated with the moult (for larvae). Adults susceptibility can be affected by changing food habits, sexual maturation.

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