

EVALUATION OF ACUTE TOXICITY OF THE ENTOMOPATHOGENIC FUNGI ON BIOLOGICAL SYSTEMS

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Abstract: For the selection of bacterial and fungal strains of biotechnological interest on biological compatibility criteria and for inclusion in integrated control schemes of diseases and pests frequently in vegetable crops protected was evaluated the acute toxicity of five species of entomopathogenic fungi at *Daphnia magna* and *Eisenia foetida*, in GLP conditions designed in the eco-toxicological facility of RDIPP Bucharest. Were tested: *Verticillium lecanii*, *Metarhizium anisopliae*, *Beauveria brongniartii*, *Beauveria bassiana* and *Isaria farinosa*. The entomopathogenic fungi tested to maximum use concentrations showed no acute effects on Daphnids and earthworms. There were no recorded immobilizations and abnormal reactions and was found that the fungus *Verticillium lecanii*, *Metarhizium anisopliae* and *Isaria farinosa* had a stimulating action on the *Daphnia magna* reproduction.

Key words: acute toxicity, biological system, entomopathogenic fungi, *Daphnia magna*, *Eisenia foetida*.

INTRODUCTION

In "LECO" testing facility have been conducted two studies for evaluate effect acute of entomopathogenic fungi to earthworms and crustaceans *Daphnia magna*. Were tested 5 species of fungi entomopathogenic: *Verticillium lecanii* at the concentration of 1.7×10^9 , *Metarhizium anisopliae* to the concentration of 7×10^7 , *Beauveria brongniartii* at the concentration of 1.9×10^8 , *Beauveria bassiana* at concentrations of 3.6×10^7 and *Isaria farinosa* in concentration 1.4×10^8 .

In the paper are presented data on the acute toxicity on five species of entomopathogenic fungi at *Daphnia magna* and *Eisenia foetida*. The research was conducted according to the GLP principles, using the biological pattern systems designed for the testing facility in RDIPP Bucharest.

MATERIAL AND METHOD

The reference material for eco-toxicological test execution is:

- OECD Guideline No.202/1992.07.17 – Acute immobilizing test in *Daphnia magna*;
- OECD Guideline No.207/1984.04.07 – Acute toxicity test on the earthworms;
- GD No. 490/ 16.05.2002;
- GLP general and specific procedures (elaborated in the Testing Facility);
- biological material: five species of entomopathogenic fungi, *Eisenia foetida*, *Daphnia magna*.

RESULTS AND DISCUSSIONS

According to the Good Laboratory Practice principles, assessment tests of acute toxicity of entomopathogenic fungi on daphnia and earthworm a comprise the following steps: the accomplishment of a frame structure in order to ensure the equipment, the production of necessary water for the tests, the achievement of the control system and soft designed for checking out the acclimation and testing rooms environmental conditions, and the elaboration of the specific operating procedures.

a) Acute toxicity of the entomopathogenic fungi on the *Daphnia magna*

The assessment of acute toxicity of the entomopathogenic fungi on *Daphnia magna*, according to the specific procedures of the biological reconstituted system developed in the laboratory were provided firstly the following:

- selection of testing species;
- breeding the testing species;
- providing of the breeding conditions;
- new born segregation;
- sensitivity testing of the tested species;

According to the Good Laboratory Practice principles the determination of acute toxicity to daphnia, respectively immobilization test was performed by static method, the testing period being of 48 hours (Fig. 1).

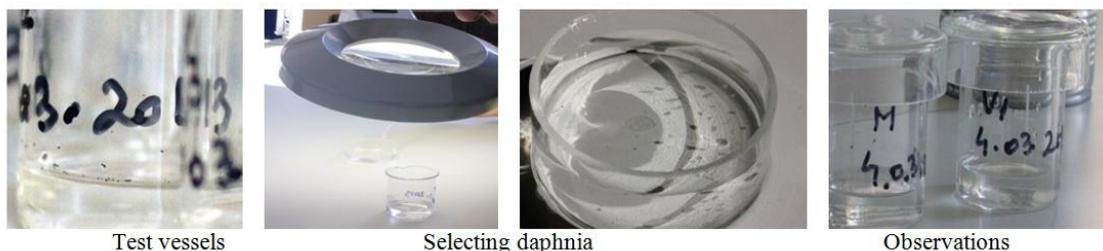


Fig.1. *Daphnia magna* - Immobilization test. Aspect during experiments

Daphnia were exposed to the concentrations of entomopathogenic fungi prepared in water, at the maximum concentration of use. Was tested and a control group exposed to the same experimental conditions. The most important experimental conditions are: the test water quality, oxygen concentration, and pH of test solution. During the test, daphnids were not fed and were guaranteed a volume of 2 ml of the test solution for each daphnia.

Daphnids were examined at 24 and 48 hours, and was registered the numbers of immobile daphnia conform with the procedure of test. Were considered immobile, daphnia that were not able to swim within 15 seconds after gentle agitation of the test vessel. Were registered also the values of dissolved oxygen concentration, pH and the temperature at the beginning and end of the test. Reference substance was potassium dichromate.

The entomopathogenic fungi tested in laboratory conditions at maximum use concentrations showed no acute effects on the *Daphnia* (Table 1). There were no recorded immobilizations and abnormal reactions and was found that the fungus *Verticillium lecanii*, *Metarhizium anisopliae* and *Isaria farinosa* had a stimulating action on the reproduction at *Daphnia*. The study was validated order that (by comparison) have been complied the criteria of quality conform with method C2 of Regulation (EC) no. 440/2008.

Table 1

Variant	Control		The species of the entomopathogenic fungi / spores concentration (UFM/ml)									
			<i>Verticillium lecanii</i> 1.7×10^9		<i>Metarhizium anisopliae</i> 7×10^7		<i>Beauveria brongniartii</i> 1.9×10^8		<i>Beauveria bassiana</i> 3.6×10^7		<i>Isaria farinosa</i> 1.4×10^8	
			24	48	24	48	24	48	24	48	24	48
Period -h/ replicative-R	24	48	24	48	24	48	24	48	24	48	24	48
R1-5 <i>D.magna</i>	0	0	0	0	0	0	0	0	0	0	0	0
R2-5 <i>D.magna</i>	0	0	0	0	0	0	0	0	0	0	0	0
R3-5 <i>D.magna</i>	0	0	0	0	0	0	0	0	0	0	0	0
R4- 5 <i>D.magna</i>	0	0	0	0	0	0	0	0	0	0	0	0
Total <i>D.magna</i> immobilized (48 h)	0		0		0		0		0		0	
Immobilization % (48 h)	0		0		0		0		0		0	
Reactions abnormal	-	-	-	-	-	-	-	-	-	-	-	-

At control group there were no recorded immobilizations and the dissolved oxygen concentration at the end of test was ≥ 3 mg / l in control and test vessels, ranging between 6.0 to 6.5 mg /L.

b) The acute toxicity of entomopathogenic fungi on the *Eisenia foetida*

To determine the toxicity of five species of entomopathogenic fungi to *Eisenia foetida*, the study was conducted according to GLP. The fungal strains tested were bred and kept in collection on medium inclined PDA at 40C. The fungi were tested at the maximum use concentrations.

The concentrations of entomopathogenic fungi prepared in water were added to an artificial soil in they were placed the earthworm adults, aged at least 2 months, with fully developed clitellum and weighing between 300 and 600 mg.

After 7 and 14 days were examined the effects of fungi entomopatogenic on the earthworm, by counting the earthworms who survived at the concentration of each fungi (Fig. 2).



Fig. 2|Aspects during 14 days observations - *Eisenia foetida* acute toxicity test

The substrate of testing was prepared according to the technical procedure, and content the peat, kaolin, clay and industrial quart sand. The substrate had a 2500 g the artificial soil with a 35% moisture. In this substrate were dispersed the fungi spores at the concentration of testing. Each concentration included four repetitions. The study was valid because the mortality of witness was below 10% ($\% M \leq 10$) at the end of test, as required by the standard.

Reference substance was chloroacetamide. Testing was conducted in glass vessels with a capacity of 1 l. During the test were observed test conditions, temperature of $200^{\circ} \text{C} \pm 2^{\circ} \text{C}$, continuous light conditions, with a light intensity of 400 to 800 lux.

The results have been demonstrating that entomopatogenic fungi tested in laboratory conditions at maximum use concentrations showed no acute effects on the earthworm (Table 2).

Table 2

The fungus tested	No. of earthworms	Earthworms mortality after 15 days				Total
		Lot 1	Lot 2	Lot 3	Lot 4	
C1 <i>B. bassiana</i>	10	0	0	0	0	0
C2 <i>-B. brongniartii</i>	10	0	0	0	0	0
C3 <i>-V. lecanii</i>	10	0	0	0	0	0
C4 <i>-I. farinosa</i>	10	0	0	0	0	0
C5 <i>-M. anisopliae</i>	10	0	0	0	0	0
Control	10	0	0	0	0	0

CONCLUSIONS

- Entomopathogenic fungi tested in laboratory conditions at the maximum concentration of use, not induced immobilization at *Daphnia* and no showed toxicity to the earthworms at recommended concentrations;
- There were no recorded immobilizations and abnormal reactions;
- The fungus *Verticillium Lecanii*, *Metarhizium anisopliae* and *Isar farinosa* had a stimulating action on the reproduction at *Daphnia*;
- The biological systems pattern for daphnia and earthworm performed in the testing facility, proved their efficacy and validity for the environmental risks assessment of the plant protection products in compliance with the Good Laboratory Practice Principles.

REFERENCES

- GOVERNMENT DECISION No. 63/2002 approving the Good Laboratory Practice Principles
GOVERNMENT DECISION No. 490/2002 methods for eco-toxicity assessment/measurement
GOVERNMENT DECISION No. 266/2006 amending and altering the Government Decision No. 63/2002
OECD No. 1- Good Laboratory Practice Principles (1997 revised)
OECD Guidelines for the Testing of Chemicals no.202/17.07.1992 – Acute immobilizing testing in *Daphnia*
OECD Guideline No.207/1984.04.07 – Acute toxicity test on the earthworms
OECD series referring to GLP and conformity monitoring