Romanian Journal for Plant Protection, Vol. IX, 2016
ISSN 2248 – 129X; ISSN-L 2248 – 129X

# THE IMPACT OF SOME MICROBIOLOGICAL PRODUCTS TOWARDS USEFUL ENTOMOPHAUNA

*Roxana Dudoiu, Daria Popa, Oana Boiu-Sicuia, Carmen Mincea**

Research-Development Institute for Plant Protection, Bucharest, Romania

*correspondence address:
Research-Development Institute for Plant Protection
8 Ion Ionescu de la Brad
013813, Bucharest, ROMANIA
Tel.:004-021-2693231
Fax: 004-021-2693239
E-mail: carmenmincea@yahoo.com

**Abstract:** Sustainable agriculture involves the use of effective plant protection products, but in the same time with low environmental impact. The application of biological products represents an ecological and efficient method for pest control by using its natural enemies. The paper presents studies regarding the impact of some biological products, two with entomopathogenic action, and the other two with fungicide activity, used as potato crop treatment for pest control. The biological material consisted in biological active bacterial biomass based on *Bacillus thuringiensis*, biological active fungi biomass based on *Beauveria bassiana*, both with entomopathogenic activity, biological active biomass based on two Bacillus strains, respectively *Bacillus safensis* Rd.b2 şi *B. subtilis* B005, with strong fungicide activity. In order to establish the ecotoxicological impact of the selected products, there were performed "in vitro" tests on *Eisenia foetida*. The experiments targeted observations regarding the medium mortalities of the test specie induced by the biological products used. The percentages of survival were 96.66% for Rdb2 treatment, 93.34% for B005 treatment, 100% for B. bassiana treatment and 63.34% for *Bacillus thuringiensis* treatment.

***Key words:*** *ecotoxicology, biopesticides, Esenia foetida, selectivity.*

## INTRODUCTION

Agriculture is facing critical challenges to ensure high crop production and quality, while preserving environmental and human health (Brussaard et al., 2010). Among pressures exerted by agriculture on the environment, synthetic plant protection products (PPPs) are of concern because they can persist in arable soils and be transferred to different compartments of the environment where they are frequently detected (Margni et al., 2002; Polyrakis, 2009). In this context, the use of natural active compounds, also known as biopesticides, may be an interesting alternative for crop protection as they are considered to be less harmful and environmentally safer (Dewhurst, 2001; Dayan et al., 2009, 2011; Cantrell et al., 2012; Seiber et al., 2014). Within the last few years, several studies highlighted the effectiveness of biopesticides for the control of different pests in various crops (Copping & Menn, 2000). Due to the diversity of biopesticide modes of action, risks of resistance emergence is considerably reduced (Copping & Menn, 2000; Dayan & Duke, 2014). Consequently, the biopesticide market has been gaining greater approval and interest for pest management (Copping & Menn, 2000; Cantrell et al., 2012; Seiber et al., 2014). To date there are only few studies describing the environmental fate and ecotoxicological impact of biopesticides on non-target organisms (NTO). In spite of their natural origin, biopesticides are active compounds used to eradicate target organisms in crops. Like other agrochemicals, once released in the soil, they may undergo a range of abiotic and biotic processes (i.e., sorption, degradation, and transport) modulating their environmental fate and conditioning their side effects on these non-target organisms.

This study presents research regarding the ecotoxicological impact of four bio-products based on biological active microbial biomass used for pest control in the potato crop.

## MATERIALS AND METHODS

The biological material consisted in four bio-products based on biological active microbial biomass, (Figure 1) respectively:

- Biological active bacterial biomass based on *Bacillus thuringiensis*, considered the natural enemy of the Colorado beetle. This specie is a Gram positive bacteria, characterized by the presence of a protein toxin with a specific mode of action through ingestion. It can be cultivated in liquid, semi-solid or solid medium. For our experiments it was used the Btt1 strain from RDIPP entomopathogenic microorganisms' collection. The bacterial inoculum was obtained after 18h cultivation of Btt1 strain in Luria-Bertani medium. After that, it was obtained the bacterial biomass to be tested, under submerse fermentation conditions, on an orbital shaker (200 rotations/minute), for 96h, at 28°C, in Luria-Bertani medium supplemented with salts (FeSO4, ZnSO4, MnSO4, MgSO4) and 0.2% glucose. It was assessed the biological titre of the bacterial biomass by counting the colony forming units (ufc/ml), which was 2.0 x 108 ufc/ml viable spores. The used dose was 2.0 x $10^8$ cfu/ml x 4l/ha, which concluded in a concentration of 8x$10^{11}$ cfu/ha.

- Biological active fungal biomass based on *Beauveria bassiana*, also with entomopathogenic activity, isolated from a natural infection, on *Leptinotarsa decemlineata.* The original strain was maintained by successive cultivation passages and revirulented periodically on the origin insect. The inoculum was obtained from a 21 days pure culture by washing in 10 ml distilled water and Tween 80 (0.01%). For disaggregation of the mycelia conidia, the suspension was vortexed with glass beads (Ø 1.7-2.1mm, ROTH), at 2000 rpm. Afterwards, the suspension was filtered through cotton wadding, and the spores' concentration was adjusted to a titre of $10^8$ conidia/ml using a hemocytometer for counting. For use in the field it is recommended a dose that will ensure $10^{11}$ conidia/ha.

- Biological active bacterial biomass based on *Bacillus safensis* Rd.b2 and *B. subtilis* B005, with fungicide activity. Two bacterial strains with antagonistic activity against phyto-pathogen agents were selected and ecotoxicological studied regarding the effect towards useful, non-target organisms. The aim of these studies was to select microbiological products, able to manage and decrease the potato crop specific pathogenic infections, with reduced toxicity towards useful fauna in order to ensure an adequate environmental protection. *B. safensis* strain Rd.b2 and *B. subtilis* strains B005 were selected from the RDIPP microorganisms' collection. These strains have high protection and crop stimulation properties. The bio-control activity consists in inhibiting the phyto-pathogenic fungi and maintaining phytosanitary equilibrium within the crop. The action mode involves plants colonization and direct competition of potential phytopathogenic fungi through cell lysis of infectious filaments and inhibition of mycelia' growth, reduction of the sporulation capacity and spores germination of pathogenic fungi for an efficient biocontrol. Among the direct mechanisms of action belonging to the selected strains there are also present compounds with antibiotic activity, volatile metabolites with fungicide activity and lytic enzymes, able to limit the growth and development of pathogenic fungi. Also, these strains have the capacity so synthetize enzymatic compounds with hydrolytic activity and plant growth stimulation potential, due to phytohormones' production involved in acceleration of cellular division and dimensions' growth, increasing cell size, uniformity and increase plant productivity.

Bacterial bioproducts based on *B. safensis* Rd.b2 and *B. subtilis* B005 designed for potato crop protection and plant growth stimulation, were obtained as an aqueous suspension. For inoculum preparation, Rd.b2 and B005 strain were cultivated on solid Luria-Bertani medium, by successive passage technique, in order to obtain pure isolated colonies. The bacterial cell concentration of the aqueous suspension was quantified with a spectrophotometer,

at 600 nm wave length. The bacterial inoculum was amended with carboxymethylcellulose 2%, a thickening and adhesion agent which facilitate the attachment of bacteria to the substrate during application of treatments.

For bacterial bioproducts use in agricultural practice it is recommended an application dose between 200 and 270l/ha of water. For field treatments, the *Bacillus* sp. bacterial bioproducts are prepared at a concentration of $10^9$ cfu/ml inoculum.

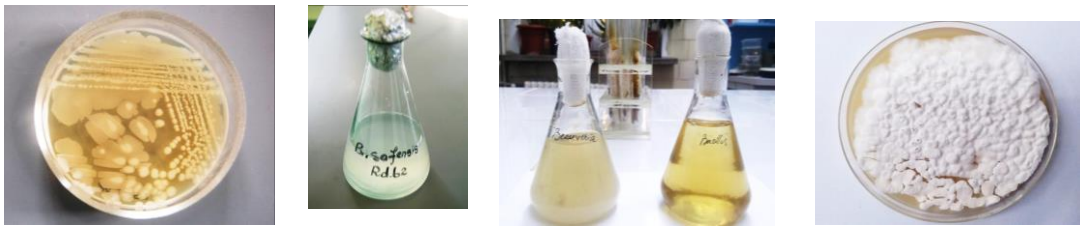

**Figure 1.** Biological products

In order to establish the ecotoxicological impact of the selected bioproducts, the tests were performed in laboratory conditions on *Eisenia foetida*. The studies were carried out using the technical procedure "Toxicity towards earthworms – artificial soil test", property of the Ecotoxicology Laboratory from Research-Development Institute for Plant Protection Bucharest (RDIPP), procedure in compliance with OECD guideline referring to chemical substances' testing. The test organism, *E. foetida* specie was procured from an authorized supplier and consisted in grown adults, with completely developed clitellum and body weight between 300 and 600 mg.

*Test principle* – adult earthworms from *E. foetida* were maintained in an artificial soil, with defined characteristics, treated with different concentrations of test substances. The content of the test jars is placed on trays after 14 (optional 7) days from treatment, and earthworms from every concentration are counted, in order to see how many survived.

Before each experiment, the earthworms were acclimatized at least 24 h, in an artificial soil, 20±20°C, in continuous light exposure with 400-800 lux intensity. The earthworms exposed to tested substances, which survived after 14 days of treatment are weighed in order to evaluate the body weight change as an indicator of sub lethal effect.

The base substrate consisted in an artificial soil with the following composition: 10% peat, with a pH of 5.5 to 6.0; kaolin 20%, preferably more than 50% kaolinite; about 69% industrial quartz sand and approximately 1% of calcium carbonate (CaCO3) powder, a pure chemical added to adjust the pH to 6.0 ± 0.5. The substrate contains the substrate base of the test, the test substance and deionized water. The water content is approximately 25-42% of the dry weight of the base substrate. The negative control variant consisted in base substrate and water.

An amount of 500 g of test substrate with different concentration of the bioproducts and 10 earthworms were placed in glass jars of 1000 l, covered with plastic caps perforated for ventilation (Figure 2). Every concentration was placed in 3 replicates. Jars were kept at 20±2°C, in continuous light conditions.

**Figure 2.** Laboratory aspects

The testing period was 14 days, and observations regarding the mortalities were made also after 7 days after treatment. The content of each jar was placed on a glass or steel tray. The earthworms were examined and it was determined the number of dead organisms (Figure 3). If the observations are performed at 7 days from treatment, after earthworms counting, the jars' content is placed back along with the surviving earthworms.

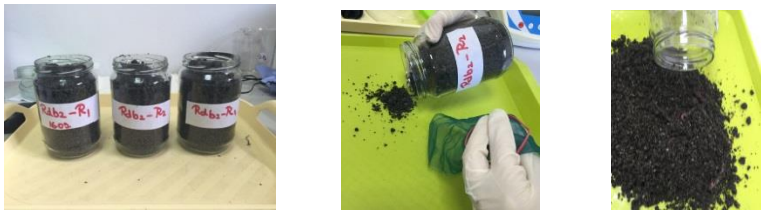

**Figure 3.** Observations regarding mortalities

Based on recorded mortalities it was calculated the mortality percentage, and based on these values it was determined the selectivity of the tested substances. The plant protection products are classified according to the mortality percentage, respectively survival percentage, in the following selectivity classes in the table 1 (Baicu, 1990):

**Table 1.** Selectivity classes

| Survival % | Selectivity | Grades on preferences scale |
|---|---|---|
| > 80 | Very selectiv | 1.0 – 0.8 |
| 80-63 | Selectiv | 0.8 – 0.63 |
| 63-37 | Medium selectiv | 0.63 – 0.37 |
| 37-20 | Low selectiv | 0.37 – 0.2 |
| <20 | Non-selectiv | 0.2 – 0.0 |

## RESULTS AND DISCUSSIONS

In the table 2 are presented the average mortality for each variant, at 7 and 14 days after treatment.

**Table 2**. Mortalities for biological tested products

| Variants | | % mortality (average) after: | |
|---|---|---|---|
| | | 7 days | 14 days |
| **Biological products** | **V1** - Rdb2 | 3.33 | 3.33 |
| | **V2** - B005 | 0 | 6.66 |
| | **V3**- *Beauveria bassiana* | 0 | 0 |
| | **V4** - *Bacillus thringiensis* | 16.6 | 36.66 |
| | **V5 - Control** | **0** | **3.33** |

The survival percentage for the tested biological products was: 96.66% for variant V1-Rdb2; 93.34% for variant V2- B005, 100% for variant V3-*Beauveria bassiana* and 83.4% after 7 days and, respectively 63.34% for variant V4- *Bacillus thuringiensis.*

## CONCLUSIONS

The microbiological tested products based on bacterial biomass Rd.b2 and B005, as well as the one based on *Beauveria bassiana* biomass have shown to be very selective towards the *Eisenia foetida* and only the biological product based on *Bacilus thuringiensis* biomass was selective. The higher mortality percentage in its case is explained by the fact that it has an ingestion mechanism of action and targets the digestive apparatus of the test specie.

From the conducted researches it can be concluded that the biological products provide protection for the detritivore macrofauna, having a low environmental impact. Due to their very good selectivity, these products can be successfully used in potato crop protection.

## ACKNOWLEDGEMENTS

This study was financed by the NUCLEU Program PN 16-29-01-02 ”*Ecotoxicological impact of the use of chemical and biological plant protection products on the environment”*.

## REFERENCES

BAICU, T., (1990). Selectivitatea substantelor chimice pentru organismele utile in combaterea integrate. Redacția de propaganda Tehnica Agricola, 20-30.

BRUSSAARD, L., CARON, P., CAMPBELL, B., LIPPER, L., MAINKA, S., RABBINGE, R. (2010). Reconciling biodiversity conservation and food security: scientific challenges for a new agriculture. Current Opinion in Environmental Sustainability, 2, 34-42.

CANTRELL, C.L., DAYAN, F.E., DUKE, S.O. (2012). Natural products as sources for new pesticides. Journal of Natural Products, 75, 1231-1242.

COPPING, L.G., MENN, J.J. (2000). Biopesticides: a review of their action, applications and efficacy. Pest Management Science, 56, 651–676.

DAYAN, F.E., CANTRELL, C., DUKE, S.O. (2009). Natural products in crop protection. Bioorganic & Medicinal Chemistry Letters, 17, 4022-4034.

DAYAN, F.E., DUKE, S.O. (2014). Natural compounds as next-generation herbicides. Plant Physiology, 166, 1090-1105.

DAYAN, F.E., HOWELL, J.L., MARAIS, J.P., FERREIRA, D., KOIVUNEN, M. (2011). Manuka oil, a natural herbicide with preemergence activity. Weed Science, 59, 464-469.

DEWHURST, I.C. (2001). Toxicological assessment of biological pesticides. Toxicological Letters, 120, 67–72.

MARGNI, M., ROSSIER, D., CRETTAZ, P., JOLLIET, O. (2002). Life cycle impact assessment of pesticides on human health and ecosystems. Agriculture, Ecosystems & Environment Journal, 93, 379-392.

POLYRAKIS, I. (2009). Environmental pollution from pesticides, in Predictive Modeling and Risk Assessment, ed Costa R., Kristbergsson K., editors. (Heidelberg: Springer Science+Business Media), 201–224.

SEIBER, J.N., COATS, J., DUKE, S.O., GROSS, A.D. (2014). Biopesticides: state of the art and future opportunities. Journal of Agricultural and Food Chemistry, 62, 11613-11619.