Romanian Journal for Plant Protection, Vol. XII, 2019
ISSN 2248 – 129X; ISSN-L 2248 – 129X

METHOD OF IMPROVING THE BIOLOGICAL POTENTIAL OF NATURAL FERTILIZERS

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Abstract: It is presented a method to obtain soil improvers substrates using microbial biomass with potential to colonize some natural fertilizers resulted as waste from agro-industrial activities; they increase the biological properties of the soil and have a direct effect on the plants, stimulating their growth, favouring the healthy development of the root system, improving the resistance to stress, thus increasing the defence against pests. It was experienced complex organic fertilizers, useful for all crops and all soil types (including unstructured, poor humus soils) and which improve the water retention capacity, soil heating and soil microbial activity. The process is based on the principle of organic farming regarding the exploitation of natural resources and offers a solution of ecological efficiency of the biological means of plant protection, by using indigenous microorganisms with bioecological potential adapted to the pedoclimatic conditions in Romania, with simple nutritional requirements, with the ability to degrade the nutrient substrate represented by organic fertilizers resulted as waste from agro-industrial activities.

Keywords: *manure, compost, Beauveria bassiaana, B. brongniartii*

INTRODUCTION

Soil is one of the most vulnerable environmental resources, but also most commonly subjected to the aggression of polluting factors, having direct and serious consequences not only on the quality of the environmental, but also on the health of people and other living beings. The most frequent factors of environmental pollution come, usually, from industry, but lately, more and more often, from agriculture. Microbial assemblages in agricultural soils are important for ecosystem services in sustainable agricultural systems, including pest control (Altieri, 1999). In this context, the beneficial microbes are often used as inoculants for production of biofertilizers, biopesticides and in bioremediation (Lugtenberg et al., 2002). Scientific research demonstrated that microbes play important roles in degrading organic wastes, agricultural wastes, replant nutrient cycling, biological fixation of nitrogen, dissolving phosphate from organic and mineral sources, stimulating plant growth by phytohormone production and nutrients uptake in soil and biological control of plant pests and diseases (Sahin, 2011; Singh et al., 2011). Significant results in this field refer to the development of biological fertilizers of agricultural interest that use bacterial or fungal microorganisms as microbial inoculants or soil inoculants; they increase the biological properties of the soil and have a direct effect on the plants, stimulating their growth, promoting the healthy development of the root system, improving the resistance to stress, thus increasing the defense capacity against the attacks of the harmful agents. Products tested as biofertilizers most often results from agricultural or industrial activities with local specificity, raw material or wastes rich in nutrient composition and bioactive compounds (e.g. banana compost, liquid waste from olive processing, livestock manure, poultry wastes, potato and carrot peels, spoiled cereal grains, bran, rice husk, rice and coconut water).

A method for the biological processing of olive mills liquid wastes for the preparation of a product suitable for agricultural use is described in Greece. This method exploits the liquid wastes as a substrate for the cultivation of dinitrogen-fixing microorganisms (*Azotobacter vinelandii*) capable of transforming the material into an organic liquid of high fertilizing and soil conditioning value (Chatjipavlidis et al., 1996). Microbial conversion of food waste inoculated with thermophilic and lipolytic *Brevibacillus borstelensis* to biofertilizer was proved to be a feasible and potential technology in the future to maintain the natural resources and to reduce the impact on environmental quality (Shu-Hsien Tsai et al., 2007). Three cellulolytic actinomycete isolates, of the genera *Micromonospora*, *Streptomyces* and *Nocardiodes*, were used as inoculant in combination with different organic amendments for rice straw composting and incorporation into soil (Hesham, 2007). Effect of five types of fertilizers (compost banana, livestock manure, poultry wastes, urea and ammonium nitrate) mixed with bioinsecticides (*Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisopilae*, margosan-O) was studied in potato culture for *Phthorimaea operculella* control (Sabbour, 2006). The effect of mixing fertilizers with bio-agents against the onion pests in the field was also presented (Sabbour & Abbass, 2006). The results of research aiming to evaluate the effects of biofertilizers and their pure cultures on phytohormones production, plant growth and yield, as well as changes in the level of phytohormones in plant leaves inoculated with biofertilizers and pure cultures used alone or in co-inoculation were presented (Asad et al., 2004). Different inoculating fungi have been studied for their beneficial effect on plant nutrition (Marschner & Timonen, 2004). A procedure to use the “bioactive mulch” (vegetal mulch suplimented with antagonistic beneficial bacteria) as an alternative to chemical fertilization of the crops is described by Constantinescu et al. (2010). There are results on the effect of some organic fertilizers on potato plants (Hermeziu, 2019). Other results in this area have been summarized in the Handbook of microbial biofertilizers (Ray, 2006). Tane et al. (2019) have presented patented results in this field: method and apparatus for producing organic fertilizer with the use of nitrogen fixing *Bacillus*, biological inoculant effective against *Aphanomyces*, biological addition product for organomineral fertilizers, plant growing media, soil conditioner and slow release bio-pesticidal and fertilizer composition, biological addition to organic-mineral fertilizers, mycoatractants and mycopesticides, process for obtaining agro-inoculant biopreparations with dormant bacteria. Studies on the toxicity effect of bioproducts based on raw-material (Păsăreanu et al., 2008), as well as on the industrial use of vine pruning residues, resulting from fructification cutting (Țenu et al., 2019) are also presented.

Production, formulation, and application of microbial biofertilizers are the important steps in the biofertilizer technology (Bashan et al. 2014). Little research attention is paid to development of optimized production schemes and formulation procedures - two of the most important steps that might ensure the efficacy of the biofertilizers - even less to application methods assuring viability of the inoculum (Vassiliev et al., 2015).

In the paper it is presented an environmentally viable method for obtaining soil improvers using microbial biomass with potential for colonization of organic substrates; the procedure is based on the principle of ecological agriculture regarding the exploitation of natural resources (native strains of *Beauveria bassiana* and *B. brongniartii*) and refers to the development of ecological products, which substantially reduce the risk of insect pests contamination, improving the soil by aerating, changing the soil structure in a positive way, increasing fertility, removing toxins, add organic matter to the soil. The purpose of this paper is to evaluate the extent to which the agrobeneficial entomopathogenic fungi having biological potential for colonization of some organic substrates, can maintain properties and ecological values that ensure them the quality of biological control agent.

MATERIALS AND METHODS

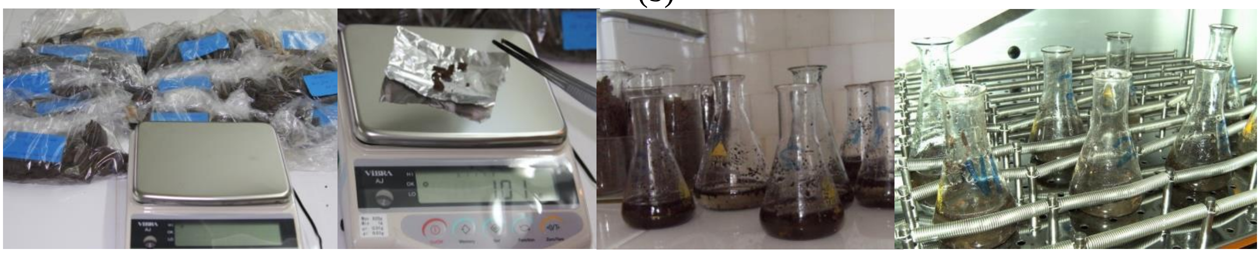
Biological material: entomopathogenic fungal biomass - *Beauveria bassiana* and *B. brongniartii* native strains.

Organic fertilizers: *compost* derived from composting of organic residues resulting in the primary vinification process and *manure* derived from fermentation of farming manure, sterilizated in autoclavable bags.

Methods for: (i) mass-multiplication of entomopathogenic fungi: solid/liquid – state fermentation and two-stage fermentation system (Andrei, 2004; Dinu et al., 2013), (ii) evaluating biological parameters (Fătu et al., 2011) and pathogenicity of fungal strains (Dinu et al., 2013), (iii) soil microbiological analysis (Fătu et al., 2016). Aspects regarding the experimental activities are presented in figure 1.

(a)

(b)



(c)

Figure 1. Aspects regarding the experimental activities: (a) Preparation of experimental soil improvers (obtaing of inoculating fungal biomass, weighing/autoclaving organic fertilizers), (b) Incorporation of soil improvers in plastic pots, (c) Preparation of soil samples for microbiological analysis

RESULTS

The soil environment constitutes an important reservoir for a diversity of entomopathogenic fungi, which can contribute significantly to the regulation of insect populations **(**Keller & Zimmermann, 1989). Of the species inhabiting the soil and belonging to Hypocreales (Ascomycota) *Beauveria bassiana* and *B. brongniartii* are considered safe (Zimmermann, 2007) and for this reason recommended for organically farmed fields.

Production of *Beauveria* sp. spores can be achieved using different methodologies, which can be classified into low input and industrial technologies. In the paper it is presented a cost-efficient production technology targeting to improve the biological potential of natural fertilizers that involves low input. The process consists in the inoculation of organic fertilization products, respectively manure and compost, with the fungal biomass resulting from the growth of *B. bassiana*, respectively *B. brongniartii* on barley grains, followed by its incorporation into the soil, in proportion of 1/200, corresponding to the doses of 20 t/ha organic fertilizer and 100 kg/ha inoculating microbial biomass. Functionality testing of the process was done under laboratory conditions. The process comprises four steps (Figure 2).

The soil improvers substrates (microbiological enriched organic fertilizers) were evaluated from a microbiological and pathological point of view. The number of colony-forming units ranged from $1.3x10^2$-$1.2x10^3$ cfu/g organic substrate, demonstrating that the manure and compost were effectively exploited as nutrient substrates, which provided fungal growth, spread and sporulation. The biological parameters of the fungal strains were quantified after re-isolating them from the fungal compositions. The results are graphically represented in the figure 3.

The germination percent of re-isolated conidia from organic substrates recorded values between 87-89% in compost and 92-93% in manure. The average daily rate of growth of the vegetative mycelium had the lowest values, for both fungal strains isolated from compost (> 12% for *B. bassiana* strain, and < 8% for *B. brongniartii* strain, respectively). The re-isolated strains from manure registered higher growth rates compared to control (synthetic culture medium): > 5% for *B. bassiana*, and > 8% for *B. brongniartii*, respectively. For the strains re-isolated from compost, the average colonies size, after 10 days post inoculation, were 7.5 cm and 3.7 cm: <35% for *B. bassiana* and <5% for *B. brongniartii*, respectively.

The virulence of the conidia isolated from manure and compost was not affected by the interaction with the organic substrates. Fungal biomass obtained in liquid medium they caused mycosis of *P. interpunctella* larvae (L_2-L_3): 87% (strains reisolated from compost) and 90% (strains re-isolated from manure).

It was found that after 90 days since the incorporation of soil improvers into pots filled with soil, in proportion of 1/200 (corresponding to the doses of 20 t/ha organic fertilizer and 100 kg/ha inoculating microbial biomass), the soil was abundantly colonized by the fungal strains, being identified mycelial hyphae both on the surface and in the depth of the substrate (Figures 4 and 5).

Biotechnological techniques for biofertilizer production and formulation are the subject of a review presented by Vassiliev et al. (2015): biotechniques that are not fully exploited as tools for biofertilizer manufacturing such as microbial co-cultivation and co-immobilization, as well as biotechnological production and combined usage of microorganisms/active natural compounds (biostimulants) such as plant extracts and exudates, compost extracts, and products like strigolactones, which improve not only plant growth and development but also plant-microbial interactions. A biotechnological procedure based on alkaline hydrolysis has been developed for effective processing of various types industrial animal wastes to convert them into a low cost high value biodegradable end product. It was demonstrated in laboratory scale experiments that the bioproduct obtained positively influenced microbial soil populations and ryegrass growth in both soils used, park soil and mine spoils (Gousterova et al., 2008). Experiments carried out in organic vineyards have shown that the microbial agroinoculants have an important role in increasing the soil's repressiveness towards phylloxera colonization (Ficiu et al., 2011), In the field of equipment for the production of bioinoculants for improving soil qualities are also concerns; major equipment of bio-organic fertilizer production is presented (https://www.fertilizermachine-solution.com/production-line/bio-organic-fertilizer-production-line.html).

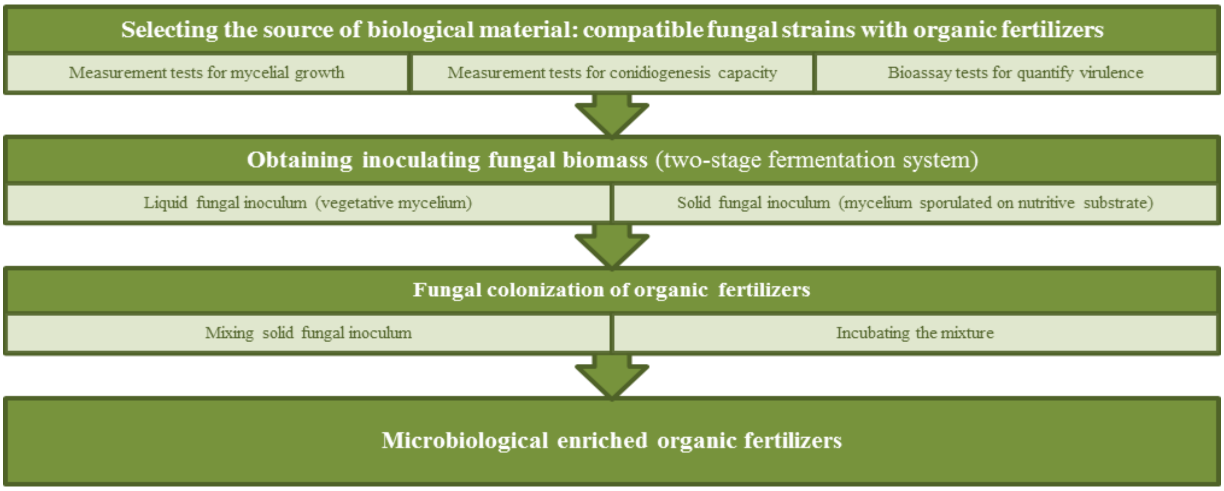


Figure 2. Stages in the process of obtaining the soil improvers

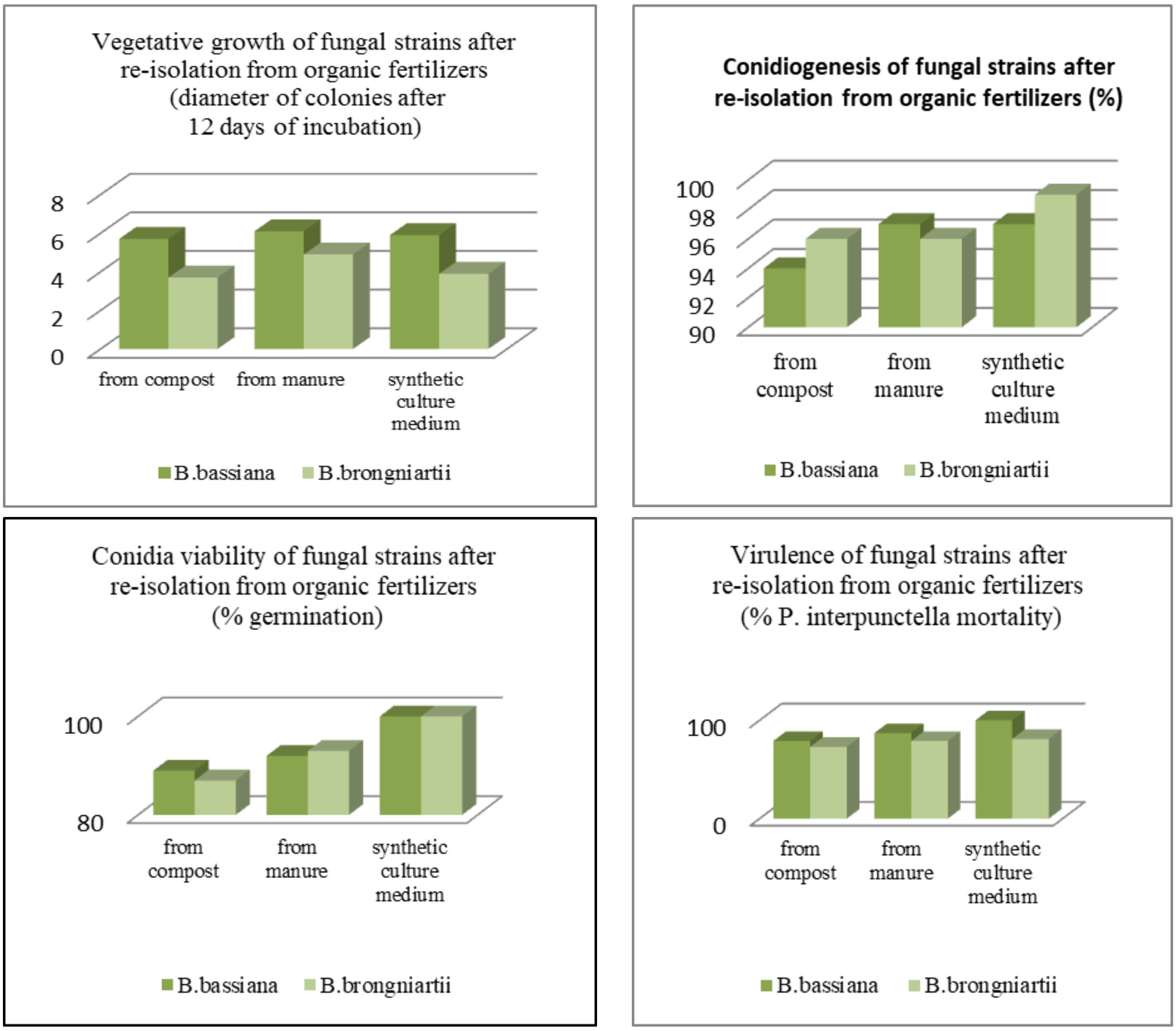


Figure 3. Biological parameters of *B. bassiana* and *B. brongniartii* strains isolated from soil improvers substrates

Manure microbiologically enriched		Compost microbiologically enriched	
B. brongniartii	*B. bassiana*	*B. brongniartii*	*B. bassiana*

Figure 4. Soil improvers substrates

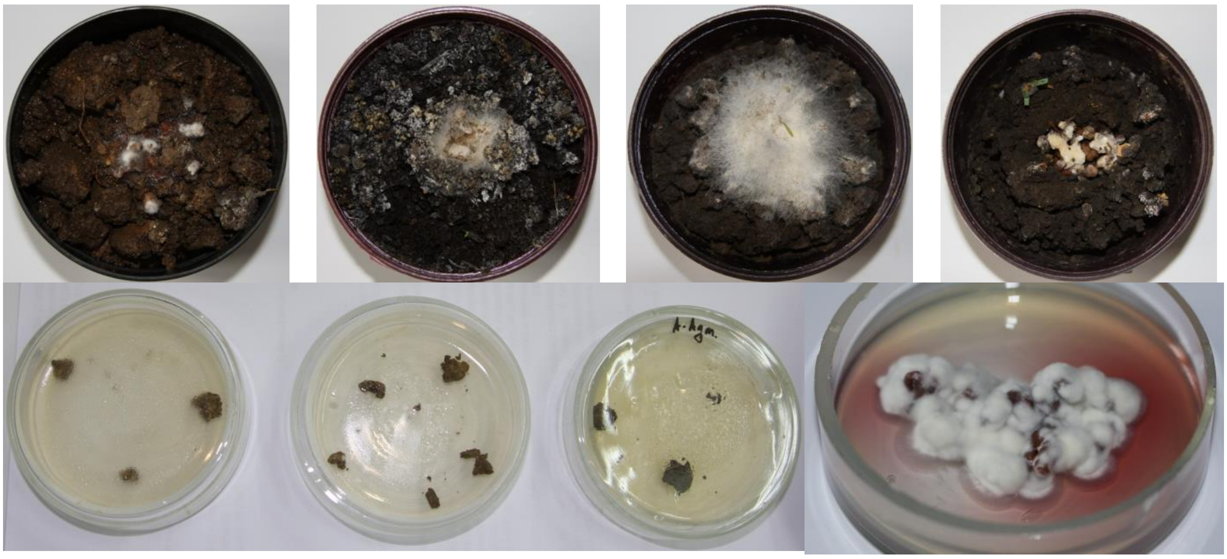


Figure 5. Soil samples from experimental pots

CONCLUSIONS

A procedure to obtain soil improvers substrates through the microbiological enrichment of some organic fertilizer resulted as wastes from agro-industrial activities was developed; it is characterized by the following steps: selection of biological control agents based on criteria of biotechnology, epizootological and pathogenicity; obtaining fungal inoculum culture in a liquid medium based on glucose, corn extract and salts, during the vegetative pre-sporulated mycelium stage; obtaining fungal biomass inoculum by insemination of vegetative pre-sporulated mycelium on nutritional organic substrate (barley grains) until complete sporulation; sterilization of organic fertilizers in autoclavable bags; making mixtures of fungal biomass with organic fertilizers; incubation of mixtures in thermostat incubator in order to obtain fungal growth and sporulation.

Examination of cultures on a standard nutrient substrate revealed vegetative growth rates and conidiogenesis that are within the normal development parameters on a synthetic medium.

The use of soil improvers substrates in proportion of 1/200, corresponding to the doses of 20 t/ha organic fertilizer and 100 kg/ha inoculating microbial biomass ensure high population levels of entomopathogenic fungi in soils.

The fact that the organic fertilizers insured an abundant saprophytic development of entomopathogenic fungi is of great practical importance because, as soil amendments, manure and compost not only adds organic matter, but it also has the advantages of less side components and good physical properties. These advantages play an important role in the organic management of agricultural crops.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-03-01/28PCCDI/2018, within PNCDI III

REFERENCES

ALTIERI, M.A. (1999). The ecological role of biodiversity in agroecosystems. *Agriculture Ecosystems and Environment*. 74, 19-31.

ANDREI, A.-M. (2004). Elemente de tehnologie pentru obtinerea unor insecticide biologice pe baza de *Beauveria bassiana.* Lucrarile celei de a 6-a Conferinta nationala pentru protectia mediului prin mijloace biologice si biotehnice si celei de a 3-a Conferinte nationale de ecosanogeneza. Ed. Pelecanus Brasov, 8-13.

ASAD, S.A., A. BANO, M. FAROOQ, M. ASLAM, A. AFZAL (2004). Comparative Study of the Effects of Bio-fertilizers on Nodulation and Yield Characteristics of Mung Bean (Phaseolus vulgaris L.). *International Journal of Agriculture and Biology*, 6, 837–43.

BASHAN, Y., DE-BASHAN, L. E., PRABHU, S.R., HERNANDEZ J.-P. (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). *Plant and Soil*, 378, 1-33.

CHATJIPAVLIDIS, I., ANTONAKOU, M., DEMOU, D., FLOURI F., BALIS, C. (1996). Bio-fertilization of olive oil mills liquid wastes. The pilot plant in Messinia, Greece. *International Biodeterioration & biodegradation*, 38, 3-4, 183-187.

CONSTANTINESCU, F., OANCEA, F., SICUIA, O.A., DINU, S. (2010). Selection of bacterial strains usefull in bioactive mulch agricultural systems. *Romanian Journal for Plant Protection*, 3, 88-93.

DINU, M. M., FĂTU, A.-C., ANDREI, A.-M. (2013). Procedee de fermentaţie microbiană utilizate pentru obținerea biopreparatelor fungice, Ed. Alpha MDN, 58 pp.

DINU, M.M., FĂTU, A.-C., CHIRILOAIE, A., ANDREI, A.-M. (2013). Procedee de crestere si utilizare a insectelor pentru testarea biologica a produselor de protectia plantelor. Ed. Alpha MDN, 40p.

FĂTU, A.-C., FATU, V., FICIU, L., ANDREI, A.-M. (2011). Metoda de testare a biocompatibilitatii dintre biopreparate fungice entomopatogene si produse organice de fertilizare. *Oferta cercetarii stiintifice pentru transfer tehnologic in agricultura, industria alimentara si silvicultura*, 19, Ed. Printech.

FĂTU, A.-C, DINU, M.M., ANDREI, A.-M. (2016). Tehnici de laborator pentru investigarea deuteromicetelor entomopatogene. Ed. A.T.U. Sibiu-Hermanstadt, 52 p.

FICIU, L., PÎRCALABU, L., CAZACU, S., GRECU, V., FĂTU, A.-C., ANDREI, A.-M. (2011). Prevenirea si diminuarea pagubelor produse de filoxera. Ed. Alpha MDN, 56 p.

GOUSTEROVA, A., NUSTOROVA, M., CHRISTOV, P., NEDKOV, P., NESHEV, G., VASILIEVA-TONKOVA, E. (2008). Development of a biotechnological procedure for tratment of animal wastes to obtain inexpensive biofertilizer (2008). *World Journal of Microbiology and Biotechnology*, 24, 11, 2647-2652. DOI: 10.1007/s11274-008-9788-1.

HERMEZIU, M. (2019). Influence of the application of organic fertilizers in association with different fungicides on the late blight (*Phytophthora infestans*) attack in potato. *Romanian Journal for Plant Protection*, 7, 8-11.

HESHAM, M. ABDULLA (2007). Enhancement of Rice Straw Composting by Lignocellulolytic Actinomycete Strains. *International Journal of Agriculture & Biology*, 106-109.
SHU-HSIEN, T., CHING-PIAO, L., SHANG-SHYNG, Y. (2007). Microbial conversion of food wastes for biofertilizer production with thermophilic lipolytic microbes. *Renewable Energy*, 32, 6, 904-915.
KELLER, S. ZIMMERMANN, G. (1989). Mycopathogens of soil insects. In: Wilding N, Collins NM, Hammond PM, Webber JF, editors. *Insect-Fungus Interactions*. Academic Press, 239-270.
LUGTENBERG, B.J.J, THOMAS, F.C., CHIN, A.W., BLOEMBERG, G.V. (2002). Microbe-plant interactions: principles and mechanisms. *Antonie van Leeuwenhoek*, 81, 373-383.
MARSCHNER, P., TIMONEN, S. (2004). Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. *Applied Soil Ecology*, 28, 23-36.
PĂSĂREANU, A., MINCEA, C., HERA E. (2008). Insecticides based on row material of vegetal origin – toxicity effect on mammals. *Romanian Journal for Plant Protection*, 1, 1-7.
RAY, M.K. (2006). Handbook of microbial biofertilizers, (M.K. Ray, ed.), 27p. Available from: https://www.k-state.edu/fungi/Greeting/Publications_files/2006%20Handbook.pdf.
SABBOUR, M.M. (2006). Effect of some fertilizers mixed with bioinsecticides on the potato tuber *Phtorimaea operculella* infesting potao in the field and store. *Pakistan Journal of Biological Sciences*, 9, 10, 1929-1934.
SABBOUR, M.M., ABBASS, M.H. (2006). The role of some bioagent mixed with some fertilizers for the control onion pests. *Journal of Applied Sciences research*, 2, 9, 624-628.
SAHIN, F (2011). Development and application of biofertilizers and biopesticides for crop production and protection. *Current Opinion in Biotechnology*, 22S. S15 – S152.
SINGH, J.S., PANDEY, V. C., SINGH, D. P. (2011). Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. Agricultural Ecosystems and Environment, 140, 339-353.
SMIRTI, V., RAVI, K.S., BRAJESH, S. (2015). Mass Production of *Beauveria bassiana* (NCIM No.1300) Fungal Spores on Cereal Grains and Agro-Industrial Residues. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 6, 1, 58-60.
ȚANE, M. C., COJANU, D., FĂTU, A.-C., ANDREI, A.-M., EUGEN, M. (2019). Methods to use the entomopathogenic fungi as bioinoculants of phytosanitary interest. *ISBA-INMA TEH, Agricultural and Mechanical Engineering*, 561-568.
ȚENU, I., CORDUNEANU, O. R., ROȘCA, R., CÂRLESCU, P., STOLERU, V., NAGHIU, A., ROMAN, C., SENILĂ, L. R., ARSENOAIA, V. (2019). Researches regarding the use of vine pruning residues, resulting from fructification cutting, for the manufacture of pellets. *ISBA-INMA TEH, Agricultural and Mechanical Engineering*, 569-576.
VASSILIEV, N., VASSILIEVA, M., LOPEZ, A., MARTOS, V., REYES, A., MAKSIMOVIC, I., EICHLER-LÖBERMANN, B., MALUSÀ, E. (2015). Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. *Applied Microbiology and Biotechnology*, 99, 12, 4983-4996.
ZIMMERMANN, G. (2007). Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology*, 17, 6, 553-596, DOI: 10.1080/09583150701309006.