EUREKA PROJECT BIO-PLANT-PROTECT AS MODEL KNOWLEDGE TRANSFER BETWEEN ACADEMIC, RESEARCH AND INDUSTRY

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EUREKA PROJECT BIO-PLANT-PROTECT AS MODEL KNOWLEDGE TRANSFER BETWEEN ACADEMIC, RESEARCH AND INDUSTRY

ABSTRACT. This paper presents an example of interdisciplinary cooperation between scientific research, education and industry to develop innovative products. Through its complex, inter-related activities, the project generates innovation in bioeconomy, in the agrifood system, developing a double action product (protection for plants and stimulation of agricultural production), as well as treatment schemes adapted to pest control and amplification of germination, specifically designed for their applicability domain (e.g. apple orchards, tomato seeds).

KEY WORDS: knowledge transfer, bio-fungicide, protection, nutrition, bioeconomy

PROIECTUL EUREKA BIO-PLANT-PROTECT CA MODEL DE TRANSFER DE CUNOȘTINȚE ÎNTRE EDUCAȚIE, CERCETARE ȘI INDUSTRIE

REZUMAT. Această lucrare prezintă un exemplu de cooperare interdisciplinară între cercetarea științifică, educație și industrie pentru a dezvolta produse inovatoare. Prin activitățile sale complexe, interconectate, proiectul generează inovație în bioeconomie, în sistemul agroalimentar, dezvoltând un produs cu dublă acțiune (protecția plantelor și stimularea producției agricole), precum și scheme de tratare adaptate combaterii dăunătorilor și amplificării germinației, special concepute pentru domeniul lor de aplicabilitate (ex. livezi de meri, semințe de roșii).

CUVINTE CHEIE: transfer de cunoștințe, bio-fungicid, protecție, nutriție, bioeconomie

LE PROJET EUREKA BIO-PLANT-PROTECT COMME MODÈLE DE TRANSFERT DE CONNAISSANCES ENTRE L'ACADÉMIE, LA RECHERCHE ET L'INDUSTRIE

RÉSUMÉ. Cet article présente un exemple de coopération interdisciplinaire entre la recherche scientifique, l'éducation et l'industrie pour développer des produits innovants. A travers ses activités complexes et interdépendantes, le projet génère de l'innovation dans la bioéconomie et dans le système agroalimentaire, en développant un produit à double action (protection des plantes et stimulation de la production agricole), ainsi que des schémas de traitement adaptés à la lutte antiparasitaire et à l'amplification de la germination, spécifiquement conçus pour leur domaine d'application (par exemple vergers de pommiers, graines de tomates).

MOTS CLÉS : transfert de connaissances, bio-fongicide, protection, nutrition, bioéconomie

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INTRODUCTION

The BIO-PLANT-Protect project is a European cooperation between research, education and industry from Romania and Poland to capitalize animal and vegetal waste in circular agriculture and to develop new bio-fungicides compositions with bivalent activity: protection against pathogens and bio-stimulation of germination and growth of plants.

The general objective of BIO-PLANT-Protect project is achieved through appropriate capitalization of vegetal waste from *Solanaceae* family and leather industry by-products – fertilizers resulting from protein extracts (collagen, keratin), associated with *Camelina sativa* oil and bio-active compounds from *Asteraceae* / *Fabaceae* family.

The innovative character is anchored in scientific priorities and European Union requirements (Directive 782/2019), regarding the minimization of harmful substances in agriculture. The concept of this project brings concrete and feasible innovative solutions by: (i) exploiting the bioactive potential of indigenous natural compounds; (ii) optimized technologies – cost-effectiveness and significant performances improvement; (iii) new prototype-amplified fungicide efficacy due to active compounds profile and ratio, for a complex spectrum of pathogens; (iv) new treatment schemes for two stages of plants' development, vegetation and seeds.

The feasibility is supported by the interdisciplinarity between partners: SC BIOTEHNOS SA Romania – research for isolation and characterization of actives, *in vitro* studies, formulation and field testing; National Research and Development Institute for Textiles and Leather, Division: Leather and Footwear Research Institute, Romania – research in capitalisation of proteinic nutrients extracts, with the involvement of doctoral young researchers and doctoral students in research activity; University of Agronomic Science and Veterinary Medicine, Bucharest, Romania – excellence in phytopathology, with the involvement of students and master's students in research activities; Łukasiewicz Research Network – Institute of Leather Industry, Łódź, Poland – recovery of collagen hydrolysates in phyto-treatments and specific analysis; Pestila II, Wolborz, Poland – pesticide production and marketing capacity at European level.

The complementary experience and expertise of the research-education-industry consortium [1] ensure a complete cycle of innovative products development and validation. Also, the work in consortium ensures the conditions for an important scientific production for dissemination and increasing the visibility of project partners.

The project generates the knowledge transfer between academic, research and industry, career development of future graduates and young graduates through involvement in applied research and the innovation capacity extension oriented to products and technologies, as well as large scale dissemination of results. Through its complex, inter-related activities, the project generates innovation in bioeconomy, in the agrifood system, developing a double action product (protection for plants and stimulation of agricultural production), as well as treatment schemes adapted to pest control and amplification of germination, specifically designed for their applicability domain (apple orchards, tomato seeds).

The main object of research in this project is to create an innovative, ambivalent bio-pesticide, using plant extracts of marigolds and fenugreek with antifungal effects [2, 3] and protein extracts of collagen and keratin recovered from animal skin by-products [4-6], with properties to stimulate seed germination and nutrition of horticultural plants in vegetation.

Previous research has demonstrated both the ability of plant extracts to prevent and combat fungal infestations in agricultural crops [7, 8], as well as the properties of animal protein extracts for biostimulation and plant nutrition [9, 10].

EXPERIMENTAL

Materials and Methods

Materials

- Residual semi-processed bovine leather for collagen extraction was collected from the leather processing pilot station of INCDTP – Division: Leather and Footwear Research Institute, chopped, and preserved by freezing.

- Wool for keratin extraction was purchased from sheep farmers and degreased at the INCDTP Division: Leather and Footwear Research Institute.

- Hydrated calcium oxide (CaO CaOH, MW = 81.371 g/mol) was purchased from Cristal R Chim SRL (Bucharest, Romania).

- Analytical grade chemical reagents, such as ammonia potassium hydroxide and oxalic acid, were purchased from Chimreactiv SRL (Bucharest, Romania).

- Alcalase 2.4 L (protease from *Bacillus licheniformis* with 2.4 U/g activity) and propionic acid were purchased from Sigma-Aldrich (Bucharest, Romania).

- Protamex® (an endo-protease from *Bacillus* spp. with 1.5 U/g activity) was purchased from Novozymes (Atasehir, Turkey).

Methods

- The methods of extracting protein hydrolysates are: thermal hydrolysis at pH=5.5-6.0 for gelatin, enzymatic hydrolysis at pH=8.0-8.5 for collagen hydrolysate, and alkaline-enzymatic hydrolysis with calcium and potassium hydroxides, at pH=8.0-9.0 for keratin hydrolysate, then settling, filtering and conditioning by drying at 60°C, cooling and grinding.

- The method of extracting antifungal compounds from plants: by stirring of the crushed vegetables at ambient temperature in a hydroalcoholic solution of ethanol 70% v/v, filtration and concentration under vacuum at 170-70 mbar at a temperature of 60°C, conditioning in butylene glycol, centrifugation for 20 min. at ambient temperature with 8000 rpm.

- Methods of characterizing protein and vegetable extracts: texture properties of gelatin (strength, consistency, elasticity, adhesion strength) were analysed according to GMIA standard methods for the testing of edible gelatin (Official Procedure of the Gelatin Manufacturers Institute of America, Inc.), using a TEX'AN TOUCH Texture Analyser, equipped with Bloom cylinder and special cylinder for CRT (Compression-Relaxation-Traction); the nanometric particle size and distribution were analysed by Dynamic Light Scattering (DLS) using a ZetaSizer Nano ZS (Malvern, UK). The determinations were made using solutions containing 1% protein; The amino acid composition of gelatin, collagen, and keratin hydrolysates was analysed by HPLC using an Amino Acid Analyzer LC 3000 (Sykam GmbH, Eresig, Germany), equipped with a polymeric cation exchanger column, post-column ninhydrin derivatization at 125°C, and photometric measurement at 570 nm. The results were monitored by Chromatography-Software ChromStar 6.0 (SCPA GmbH, Bremen, Germany) and were reported as a means of triplicate determinations.

- The method of studying germination is based on calculation of the mean standard deviation (±) and the relative standard deviation (RSD). The statistical analysis was carried out with the help of Microsoft Excel 2019. The results were interpreted by applying the Paired Two Sample for Means test to compare the phytostimulation activity of the studied protein hydrolysates. A value of $p < 0.05$ was considered to be statistically significant.

- The method of testing the fungicidal action of the extracts was the diffusion method with filter paper discs impregnated in the test substance and placed on the PDA medium (potato-dextrose-agar from Carl Roth GmbH +Co), solidified by cooling at 45°C. The effectiveness of the solution was assessed by manually measuring the growth of the inoculum around the discs.

RESULTS AND DISCUSSIONS

So far, models and technologies have been developed and experimented for the

extraction of proteins from leather by-products and the extraction of active plant principles with antifungal properties. Protein extracts, conditioned and characterized, were made at the laboratory and pilot scales: gelatin, collagen hydrolysate, keratin hydrolysate and their combinations; vegetable extracts with content strictly determined by active principles: flavones and α-terthiophene in the marigold extract; diosgenin in fenugreek extract. The effectiveness of combinations of protein extracts was evaluated through germination tests of bell pepper and tomato seeds treated with protein extracts. The evaluation of plant extracts was carried out from the point of view of the cytotoxic effect on human cells. Formulas of biopesticides with antifungal and fertilization activity have been created, which are being evaluated through tests in real conditions. The results were disseminated through the publication of articles in journals with international visibility and presentation in international conferences.

Figure 1 shows the experimental protein extracts in a conditioned form: gelatin extracted from hide byproducts, collagen hydrolysate extracted from residues from the gelatin extraction process and keratin hydrolysate extracted from sheep wool, after it has been degreased.

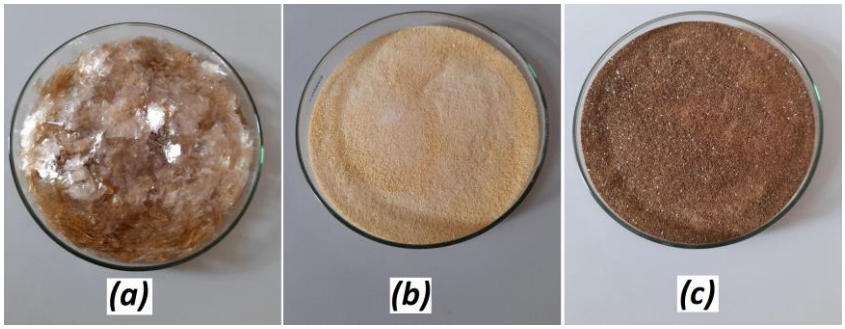

Figure 1. Protein extracts: (a) gelatin; (b) collagen hydrolysate; (c) keratin hydrolysate

The protein extracts were analysed to determine the most important properties.

Figure 2 shows the texture parameters of the gelatin, determined according to the GMIA standard methods for testing edible gelatin (Official Procedure of the Gelatin Manufacturers Institute of America, Inc.), and gelatin hardness, respectively, by Bloom test and consistency, elasticity and adhesive strength by CRT test (Compression-Relaxation-Traction).

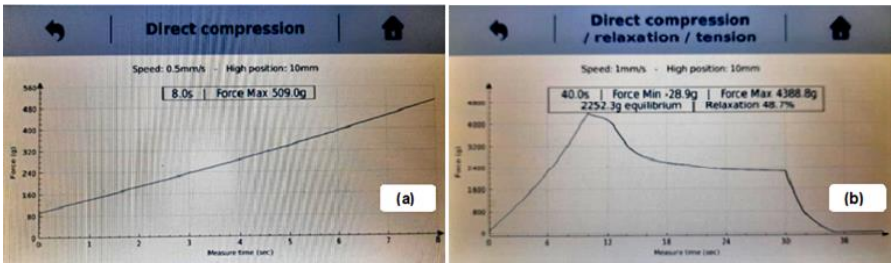

Figure 2. Gelatin texture: (a) Bloom test; (b) CRT test

Analytical data according to the Bloom test indicate that the gelatin has a high strength, expressed by the maximum force of 509 g. The consistency evaluated by the CRT (contraction-relaxation-tension) test is defined by the maximum force of 4388.8 g, elasticity is inversely proportional to the relaxation of 48.7% and the adhesion force is defined by the minimum force of -28.9 g.

As a top-level gelatin (strength over 300 g in the Bloom test), there is no risk of coagulation at low temperatures, when preparing mixtures of protein additives, or at low dilutions, but it ensures a high content of

large polypeptides to induce a good adhesion for surface applications and the delayed release of protein components with low molecular masses, amino acids and oligopeptides, respectively.

The amino acid content of collagen and keratin hydrolysates was analysed, and synthesized in the graphs in Figure 3, while the nanometric particle size distribution is presented in the histograms in Figure 4.

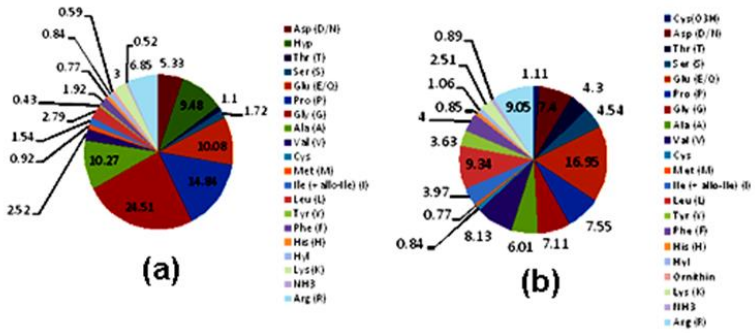

Figure 3. Amino acid content in: (a) collagen hydrolysate (b) and keratin hydrolysate

It is obvious that there are significant differences between the collagen hydrolysate and the keratin one, from simple to double or even triple, such as the case of proline, glycine, alanine more abundant in collagen hydrolysate, or the case of threonine, serine, valine, leucine, tyrosine, more abundant in keratin hydrolysate. Collagen is characterized by the presence of hydroxyproline, and in the case of keratin, it is the presence of cysteine that brings sulfur, a phytonutrient that can significantly improve the crop yield and quality.

The presence of nanometric particles and their distribution in the hydrolysates is highlighted by the dynamic measurement technique of the reflected light that crosses the diluted solutions, as shown in Figure 4. For the protein extracts, nanometric particles are associated with the presence of free amino acids and oligopeptides especially in hydrolysates, the only ones capable of penetrating cell membranes and stimulating biological processes in seeds and plants.

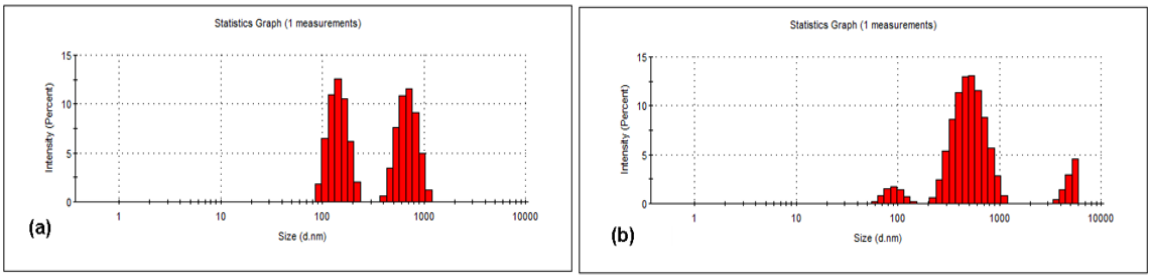

Figure 4. Particle size distribution in: (a) collagen hydrolysate; (b) and keratin hydrolysate

The light intensity measurements reflected in the hydrolysate samples indicate both small and medium particle populations, in the 100-1000 nm range, as well as particle populations with sizes smaller than 100 nm and larger than 1000 nm, especially in the keratin hydrolysate. In the composition of collagen hydrolysate, we find a population of 1.8% particles of 10-100 nm, a predominant population of 97% particles of 100-1000 nm and a population of 1.2% particles of 1000-6000 nm. In the composition of keratin hydrolysate, we find a population of 4.2% particles of 10-100 nm, a population of 85.6% particles of 100-1000 nm and a population of 10.2% particles of 1000-6000 nm.

The protein extracts made were associated with each other to potentiate the nutritional and systemic protection effects of plants in various periods of vegetation and

two types of protein combinations were made: (I) collagen-keratin protein combination, with gelatin, collagen hydrolysate and keratin hydrolysate; (II) collagenic combinations, with gelatin and collagen hydrolysate, in two variants, with which bell pepper and tomato seeds were treated at concentrations of 1%, 3% and 10%, which were subjected to a germination test, as shown in Figure 5.

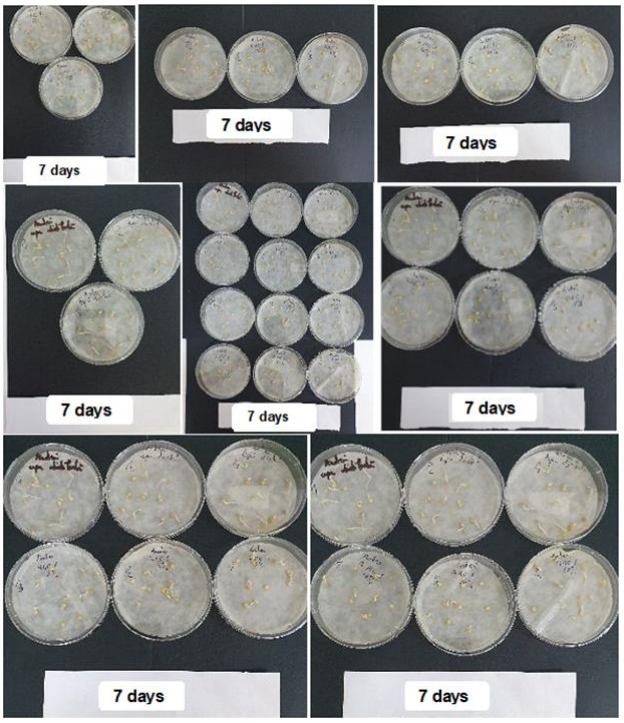

Figure 5. Testing the germination of bell pepper seeds treated with collagen and keratin combinations

The results were analysed by determining the following indicators: germination percentage, relative seed germination index (RSG), relative root growth index (RRG) and germination index (Gi). The protein combinations tested showed a phytostimulating effect on tomato seeds, when applied at a concentration of 1%, the best values being obtained for the combination with keratin content. The pepper seeds proved to be more sensitive to the phytochemical substances in the protein combinations, this being observed after the delayed germination process of the seeds, inhibiting the growth and development of the roots at the same time.

The plant extracts of French marigold and fenugreek were tested for their antifungal activity against the pathogenic fungi of the species *Botrytis cinerea*, *Monilinia* spp., *Fusarium* spp. and *Alternaria* spp.

Figure 6 shows the fungicidal action of vegetable extracts on the growth of *Monilinia* spp. pathogens at 3, 6, 9 and 12 days after incubation.

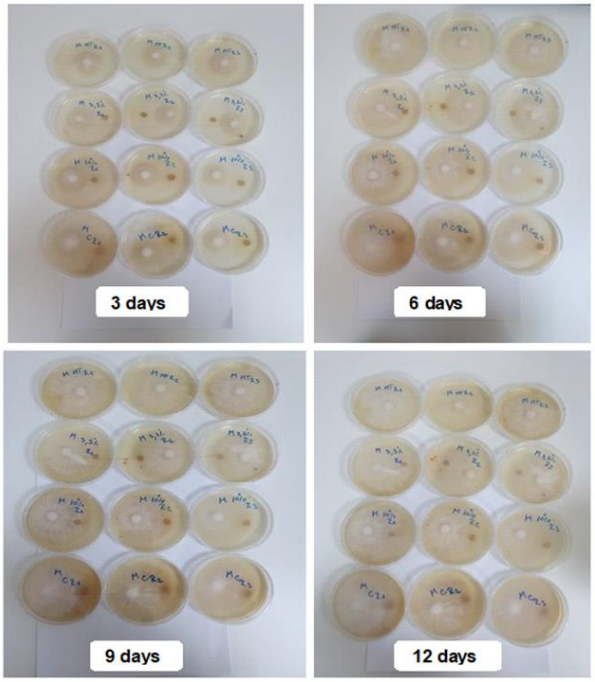

Figure 6. The fungicidal action of vegetable extracts on the growth of *Monilinia* spp. Pathogens

The highest value of the effectiveness of the French marigold extract conditioned in butylene glycol was recorded against *Botrytis cinerea* with E=66.9%, 6 days after incubation. However, with 10% aqueous extract solutions, the highest efficacies were recorded against *Botrytis cinerea* at 6 and 9 days, with close values E=73% and E=72.9%, followed by *Fusarium* spp. with E=72.6% after 6 days and E=66.4 9 days after incubation. Compared to *Alternaria* spp. and *Monilinia* spp., the highest inhibition percentage values were recorded after 9 days of incubation with E=65.7% for *Monilinia* spp. and E=63.2% for the pathogen *Alternaria* spp.

The highest values of the effectiveness of the fenugreek extract were E=81.16% for *Botrytis cinerea* and E=71.51% for *Monilinia* spp., while for the pathogens *Fusarium* spp. and *Alternaria* spp. the effectiveness was over 68% and 64%, respectively.

Two of the variants of biopesticide formulas with antifungal activity and plant nutrition, made for testing in real orchard and field culture conditions, are presented in Figure 7.

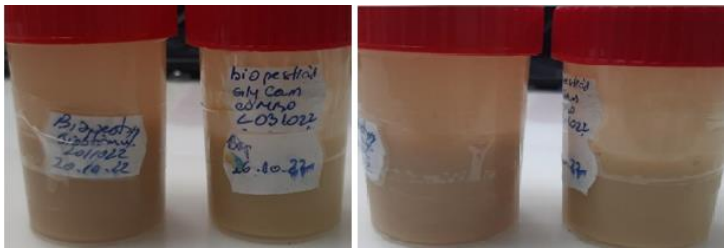

Figure 7. Variants of biofungicide for testing in real conditions of horticultural crops

One of the variants represents the association between the protein combination based on collagen and keratin, hydrolysed extract from fenugreek seeds conditioned in butylene glycol, camelina oil and extract from *Solanum lycopersicum*, and the second variant represents the association between extract from French marigold flowers conditioned in butylene glycol, protein combination based on collagen, camelina oil and *Solanum lycopersicum* extract.

The results of the application tests of the biofungicide test variants in real conditions of horticultural crops will be available for presentation in a future article, dedicated exclusively to biofungicide

applications with protective and nutritional effects in horticultural crops.

CONCLUSIONS

The project has achieved the scientific and technical objectives up to the present moment.

The complementary experience and expertise of the research-education-industry consortium ensure a complete cycle of innovative products development and validation.

The BIO PLANT Protect project created the conditions for the launch on the market of a new biopesticide with additional properties for biostimulation and nutrition in horticulture.

The visibility of the research consortium increased by disseminating the results (joint publications and presentations).

Acknowledgements

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REFERENCES

1. https://eertis.eu
2. Villaverde, J.J., Sandín-España, P., Sevilla-Morán, B., López-Goti, C., Alonso-Prados, J.L., Biopesticides from Natural Products: Current Development, Legislative Framework, and Future Trends, *BioResources*, **2016**, 11, 2, 5618-5640, https://doi.org/10.15376/biores.11.2.Villaverde.
3. Suteu, D., Rusu, L., Zaharia, C., Badeanu M., Daraban, G.M., Challenge of Utilization Vegetal Extracts as Natural Plant Protection Products, *Appl Sci*, **2020**, 10, 24, 8913, https://doi.org/10.3390/app10248913.
4. Hu, J., Xiao, Z., Zhou, R., Deng, W., Wang, M., Ma, S., Ecological Utilization of Leather Tannery Waste with Circular Economy Model, *J Clean Prod*, **2011**, 19, 221-228, https://doi.org/10.1016/j.jclepro.2010.09.018.
5. Zheng, K., Xu, T., Qian, C., Liang, M., Effect of Low Molecular Weight Fish Protein Hydrolysate on Growth Performance and IGF-I Expression in Japanese Flounder (*Paralichthys olivaceus*) Fed High Plant Protein Diets, *Aquac Nutr*, **2014**, 20, 4, 372-380, https://doi.org/10.1111/anu.12090.
6. Alao, B.O., Falowo, A.B., Chulayo, A., Muchenje, V., The Potential of Animal By-Products in Food Systems: Production, Prospects and Challenges-Review, *Sustainability*, **2017**, 9, 1089, https://doi.org/10.3390/su9071089.
7. Cristea, S., Manole, M.S., Zala, C., Jurcoane, S., Dănăilă-Guidea, S., Dumitriu, B., Temocico, G., Călinescu, M., Olariu, L., Antifungal Effect of Some Steroidal Glycoalkaloids on *Monilinia fructigena* (Aderh. & Ruhl.) Honey Fungus, *Lucrări Ştiinţifice*, Agronomie series, USAMV Iasi, **2017**, 60, 2, 65-68.
8. Morcia, C., Tumino, G., Ghizzoni, R., Bara, A., Salhi, N., Terzi, V., *In Vitro* Evaluation of Sub-Lethal Concentrations of Plant-Derived Antifungal Compounds on Fusaria Growth and Mycotoxin Production, *Molecules*, **2017**, 22, 8, 1271, https://doi.org/10.3390/molecules22081271.
9. Niculescu, M.-D., Epure, D.G., Lasoń-Rydel, M., Gaidău, C., Gîdea, M., Enăscuță, C., Multifunctional Biocomposites Based on Collagen and Keratin with Properties for Agriculture and Industry Applications, *Eurobiotech J*, **2019**, 3, 3, 160-166, https://doi.org/10.2478/ebtj-2019-0019.
10. Corte, L., Dell'Abate, M.T., Magini, A., Migliore, M., Felici, B., Roscini, L., Sardella, R., Tancini, B., Emiliani, C., Cardinali, G., Benedetti, A., Assessment of Safety and Efficiency of Nitrogen Organic Fertilizers from Animal-Based Protein Hydrolysates – A Laboratory Multidisciplinary Approach, *J Sci Food Agric*, **2014**, 94, 2, 235–245, https://doi.org/10.1002/jsfa.6239.