NEW BIOPLASTIC MATERIALS WITH BIOLOGICALLY ACTIVE PROPERTIES UNDER SIMULATED COMMERCIAL USAGE CONDITIONS

RUGINESCU Robert, BATRÎNESCU-MOTEAU Costin, NEAGU Simona, LUCACI Anca Ioana, COJOC Roxana, PODOSU (VLAD) Aurelia, ENACHE Mădălin

Abstract. The obtained data demonstrated that the investigated bioplastics, comprised of a polymer matrix based on polylactic acid supplemented with antimicrobial and antioxidant compounds, exhibited a generally patchy appearance with air bubbles present in some samples. Additionally, insoluble particles of reddish-brown colour, varying in terms of agglomeration, were observed. The samples displayed varying degrees of water absorption, ranging from 0.6% to 3.6%. The physical structure of bioplastics was also reflected in the migration of biologically active components under simulated usage conditions, resembling real-world scenarios. Notably, a relatively high migration was observed from the material's surface enriched with cinnamon. Furthermore, the study results indicated that the tested formulations inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica*. Based on these results, we could expect an extended shelf life of the packaged products by at least 24 to 48 hours compared to conventional packages. Ecotoxicity assessments revealed that the survival rate of the rotifer *Brachionus calyciflorus* was, in most cases, 100%. The data suggested that the tested bioplastics posed minimal risk to terrestrial and aquatic ecosystems, indicating low environmental impact.

Keywords: bioplastics, antibacterial activity, ecotoxicity, water absorption.

Rezumat. Materiale bioplastice noi biologic active în condiţii simulate de utilizare comercială. Datele obţinute demonstrează că bioplasticele investigate, reprezentate de o matrice polimerică bazată pe acid polilactic şi suplimentată cu compuşi care au activitate antimicrobiană şi antioxidantă, se caracterizează printr-un aspect general neomogen cu conținut de bule de aer în unele probe şi prezenţa unor particule insolubile de culoare brun-roșcat, cu grad variabil de aglomerare. Eșantioanele prezintă grade diferite de absorbţie a apei cuprinse între 0,6% şi 3,6%. Structura fizică a bioplasticului se reflectă şi în migrarea componentelor biologic active din compoziţia acestuia în condiţii simulate de utilizare, similare celor reale. De remarcat că s-a observat o migrare relativ ridicată de la suprafața materialului îmbogățit cu scorțișoară. În plus, rezultatele studiului demonstrează că recepturile testate au efect inhibitor asupra creșterii *Staphyloccocus aureus*, *Escherichia coli* și *Salmonella enterica.* Pe baza acestor rezultate, am putea să ne așteptăm la o creștere a termenului de valabilitate a produselor ambalate cu cel puțin 24 până la 48 de ore în comparație cu ambalajele convenționale. Testele de ecotoxicitate au demonstrat că rata de supraviețuire a rotiferului *Brachionus calyciflorus* este în majoritatea cazurilor de 100%. Datele obținute demonstrează că bioplasticul testat prezintă risc foarte redus asupra mediului înconjurător atât în ecosisteme terestre cât şi acvatice.

Cuvinte cheie: bioplastic, activitate antibacteriană, ecotoxicitate, absorbţia apei.

INTRODUCTION

Current concerns regarding the consequences of the agro-industrial use of various material categories, including plastics, primarily for the environment and human health, have led to the development of appropriate management policies aiming to replace conventional materials with new ones (MERCIU et al., 2009; NEAGU et al., 2014) that have reduced impacts and risks on nature. Bioplastic materials such as polyhydroxyalkanoates (PHA), polylactic acid (PLA), or polybutylene succinate (PBS) are currently being successfully used in various industrial sectors as substitutes for conventional plastics (NANNI et al., 2021). However, the biodegradability of biomass-derived plastic materials (obtained from algae or plant materials) is not guaranteed, as they contain chemical components of the source material. In contrast, biodegradable bioplastics are obtained under conditions demonstrating their ability to decompose under specific microclimatic conditions generated by the presence of microorganisms, humidity, and a characteristic temperature range (MAZHANDU et al., 2020).

Since bioplastic material represents a mixture of compounds, there is a possibility that some of these may have undesired effects on water or soil, requiring further studies in this field. For bioplastics used in agriculture that eventually reach the soil, strains of microorganisms belonging to the *Bacillus* and *Aspergillus* genera have been identified. These microorganisms, through their enzymatic equipment, contribute to the degradation of bioplastics to mineralization (NASROLLAHZADEH et al., 2021). On the other hand, in aquatic environments, the long polymeric chains of plastic materials are initially broken down abiotically by various physicochemical factors, such as UV radiation or salinity, into smaller fragments that can be taken up by bacterioplankton for use as nutrients (FOLINO et al., 2020).

In industrial facilities providing controlled conditions of temperature, humidity, and the presence of specific microorganisms, the degradation of bioplastic materials occurs with minimal environmental impact (AWASTHI et al., 2022). However, in natural environments lacking the controlled conditions of industrial plastic waste processing, the biodegradation of bioplastic materials is not as efficient. Natural terrestrial or aquatic ecosystems are characterized by conditions where the biodegradation process of bioplastics, even when derived from plants, progresses much more slowly. Polymeric matrices based on polylactic acid (PLA), obtained from renewable sources such as corn flour, are efficiently processed in industrial conditions, whereas, in natural settings, biodegradation is significantly slowed down (KALITA et al., 2021).

The biodegradation of bioplastic materials is a complex, dualistic process, considering that it releases nutrients into the environment with beneficial effects on ecosystems by stimulating both the nutrient cycle and microbial development. However, on the flip side, this process may favour eutrophication, negatively impacting aquatic life. The intricate nature of this process can affect ecosystem dynamics and microbial diversity due to competitive interactions within microbial communities (ADHIKARI et al., 2016; HAN et al., 2021).

According to literature data (KHALID et al., 2022), an estimated annual quantity of approximately 12 million tons of plastic finds its way into the environment, particularly aquatic ecosystems. Large amounts of microplastics, exceeding 260 thousand tons, are thus thought to be present in the environment in various forms, some of which are considered resistant over very long periods (KHALID et al., 2023). In this context, alternatives to conventional plastic materials are crucial. These alternatives should be environmentally friendly and contribute to the efficient utilization of natural resources.

The primary objective of this study was to highlight the antimicrobial properties and low ecotoxicity of specific types of bioplastics based on a main composition of polylactic acid, supplemented with essential thyme oil and grape pomace extract as a source of resveratrol and flavonoids. The grape pomace extract was derived from the secondary stream of raw material processing in winemaking. These bioplastics are designed to produce biodegradable packaging within the food industry. Additionally, the study demonstrated that these novel packaging materials can extend the shelf life of packaged food products by more than 24 to 48 hours from the time of packaging.

MATERIALS AND METHODS

Samples Composition (Formulations) – Five bioplastic formulations were prepared by S.C. MEDACRIL S.R.L. (Mediaş, Romania) and were tested in the present study. Details about their constituents are provided in Table 1.

Table 1. The composition of the five bioplastic samples tested in the present study. PLA = polylactic acid.

Formulation	**Face**	**PLA** (%, w/w)	**Grounded grape pomace** (%, w/w)	**Essential oil** (%, w/w)	**Other constituents** (%, w/w)
1	A	70	10	-	20
	B	75	2	Thyme 2	21
2	A	70	11	-	19
	B	75	1	Thyme 3	21
3	A	70	12	-	18
	B	75	2	Thyme 2	21
4	A	70	8	-	22
	B	75	2	Thyme 2	21
5	A	70	7	-	23
	B	75	2	Cinnamon 2	21

Imaging Characterization of Samples - A total of 5 samples of biocompatible polymeric blend, each measuring 4×6 cm with a thickness of 2 mm, taken from double-faced material, were visually analysed using an AxioZeiss Imager.M2m optical microscope at 5x magnification. Representative images for each side of the investigated material were selected.

Water Absorption Capacity Determination - The method described by Navasingh (NAVASINGH et al., 2023) with some modifications was applied, following the immersion procedure for 24 hours according to ASTM Standard 570 - 98. The material was fully immersed in distilled water at 23 °C, supported by the vessel wall. 24 hours later, the samples were removed from the water, blotted with filter paper to remove residual water, and then accurately weighed to 0.001 g. The percentage increase in weight due to water absorption was calculated using the formula C_g (%) = $(G_{um} – G_{us})/G_{us}×100$, where C_g represents the percentage increase in weight, G_{um} is the wet weight, and G_{us} is the dry weight. Test specimens were processed from the sample to ensure smooth edges and the absence of cracks.

Migration Tests - The samples were weighed and immersed in 100 mL of simulated packaged food product (3% acetic acid) for this test. After 10 days of incubation at 40 °C, they were extracted from the simulant, dried at 105°C for 1 hour, and reweighed, thus deducing the mass of migrated components (Test 1). Additionally, the mass of migrated components was determined by distributing the simulant onto glass plates, evaporating it at 90-95°C on an electric hotplate, fully drying the samples in an oven (105°C, 30 min), and reweighing (Test 2).

Determination of Antimicrobial Potential - Using the liquid culture method, the antimicrobial potential was assessed against the reference strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Salmonella enterica* ATCC 14028. To this end, the samples were sterilized by exposure to UV radiation on each side for 30-40 minutes. Subsequently, they were distributed into 12 mL of nutrient broth culture medium (10 g/L peptone, 1 g/L meat extract, 2 g/L yeast extract, 5 g/L NaCl, pH 7) inoculated with 60 µL of bacterial inoculum with a turbidity equivalent to McFarland standard 0.5, corresponding to a bacterial density of $1\text{-}2×10^8$ Colony Forming-Units (CFU)/mL. The inoculum density in each sample was thus $5×10^5$ CFU/mL (McFARLAND, 1907; ARYAL, 2021). Incubation was carried out at 37 °C for 24 hours under agitation at 140 rpm. After the incubation period, a volume of 100 µL from each sample (properly diluted in sterile physiological saline) was spread on the surface of solid nutrient agar culture medium (10 g/L peptone, 1 g/L meat extract, 2 g/L yeast extract, 5 g/L NaCl, 18 g agar; pH 7.0), and the plates were incubated at 37 °C for 24 hours. The number of Colony Forming Units (CFU)/mL was quantified in the tested samples compared to the positive control (without sample), and the percentage reduction in bacterial growth was calculated using the formula: 100-[100 × (CFU_{sample}/$CFU_{control}$)].

Ecotoxicity tests - To assess the ecotoxicity of the investigated samples, the rotifer *Brachionus calyciflorus* (Microbiotests, Belgium) was employed. Dormant eggs were distributed in a 10 mL EPA solution (96 mg/L NaHCO3, 60 mg/L CaSO4, 60 mg/L MgSO4, and 4 mg/L KCl) in Petri dishes and incubated for 16-18 hours at 25 °C, under continuous illumination to promote hatching. Plastic material samples were distributed in 15 mL EPA solution in Petri dishes, where 15 viable specimens of *Brachionus calyciflorus* were added and incubated at 25 °C for 24 hours in darkness. Viable rotifers in each dish were counted, determining whether the samples led to a loss of viability by evaluating mobility for 5 seconds. The absence of mobility after this interval is recorded as a negative result (non-viable rotifer).

RESULTS AND DISCUSSION

The analysed material was a polymeric matrix based on polylactic acid supplemented with additives containing antimicrobial and antioxidant compounds, specifically thyme essential oil and grape pomace extract (Table 1). The grape pomace extract was derived from the secondary stream of processing raw materials in the wine industry, serving as a source of flavonoids and resveratrol (COJOC et al., 2019). The optical microscopy analyses for both faces of the investigated plastic samples highlighted a generally non-uniform appearance with air bubbles in some samples. Most analysed samples contained insoluble particles of reddish-brown colour with a variable degree of agglomeration (Fig. 1). Some literature findings demonstrated that the use of such raw materials for the production of value-added products contributes to the development of new biotechnologies for environmental conservation and protection (COJOC et al., 2019; PODOSU et al., 2023).

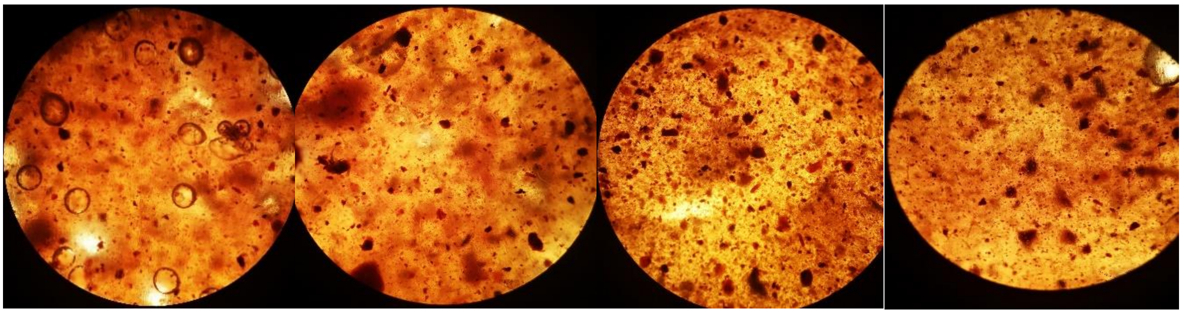

Figure 1. Microscopic appearance of investigated bioplastic samples showing non-uniform appearance and the presence of air bubbles and reddish-brown particles.

To improve the suitability of the tested bioplastic for industrial applications, optimal solutions in this context may involve enhancing homogeneity, finely grinding the compound responsible for biological properties (grape pomace), and conducting processing at lower temperatures.

The water absorption capacity of the investigated bioplastic material samples was evaluated using the standard testing method D570−98. This method determines the relative rate of water absorption by plastic materials when immersed in water (Fig. 2).

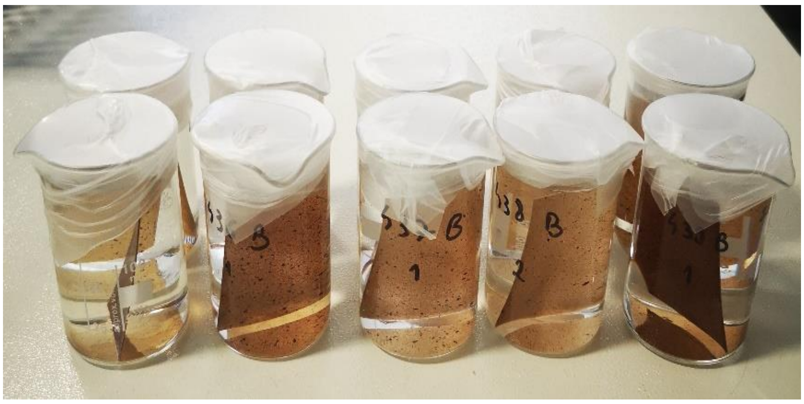

Figure 2. Bioplastic material samples tested for absorption capacity immersed in distilled water.

The testing method serves two primary functions: firstly, as a guide to the proportion of water absorbed by a material, thus providing insights into relationships between humidity and electrical or mechanical properties, dimensions, or appearance. Secondly, it functions as a control test for the uniformity of the tested product, offering guidance on the effects of water exposure or humidity on these properties. Ideal liquid diffusion in polymers is a function of the square root of the immersion time, and the time to saturation strongly depends on the thickness of the sample. The moisture content of a plastic material is intricately correlated with properties such as electrical insulation resistance, dielectric losses, mechanical strength, appearance, and dimensions. The impact on these properties due to changes in moisture content resulting from water absorption largely depends on the exposure method (immersion in water or exposure to high temperatures), the component's shape, and the plastic's inherent properties.

The results obtained for the investigated samples, tested in rectangles with dimensions of 4×6 cm, were expressed as percentages (Fig. 3) and showed varying degrees of water absorption ranging from 0.6% to 3.6%. The water absorption capacity correlated with the samples' predominantly non-uniform and variable appearance, highlighted by optical microscopy tests (Fig. 1). Additionally, the presence of air bubbles and particles that imparted the biological properties of the bioplastic, contributing to its roughness, influenced this capacity. As evident from the data presented in Figure 3, the formulation assigned to sample number 2 exhibited the highest water absorption capacity, while the formulation in sample 5 demonstrated the lowest water absorption capacity.

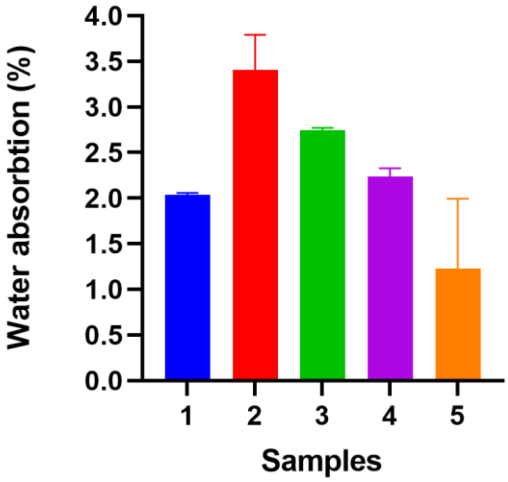

Figure 3. Water absorption capacity of the five samples/formulations – face B.

A) **B)**

Figure 4. Bioplastic material samples distributed in Berzelius beakers before (**A**) and after (**B**) immersion in the simulant.

A) **B)**

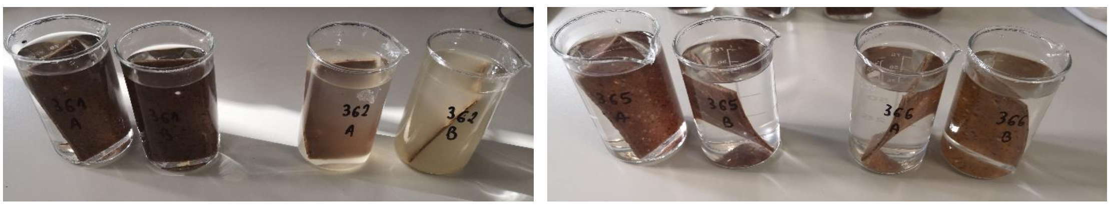

Figure 5. Bioplastic material samples after 10 days of incubation at 40 °C.
Increased turbidity of the simulant suggests a significant amount of migrated components.

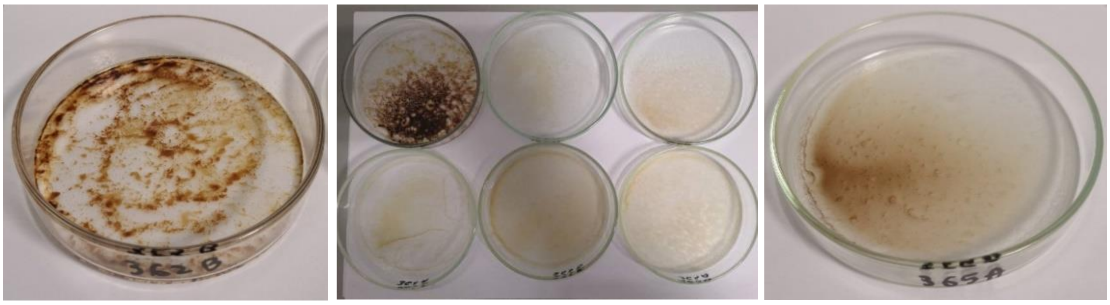

Figure 6. Evaporation of the simulant after drying in an oven at 105 °C for 30 minutes.

The results related to migrating biologically active components from the bioplastic composition under simulated usage conditions, similar to real ones, showed variations among samples, with notable differences between the two conducted tests (Figs. 4, 5, and 6). In the first migration test (Fig. 5), the highest migration rate was recorded for samples 4 and 5, while the lowest was observed for sample 1 (Fig. 7). In the second test (Fig. 6), the highest rate was observed for sample 5, and the lowest for sample 1 (Fig. 8). While the deduced mass of the migrated components in test 1 differed significantly from the dry residue mass in test 2, both tests consistently indicated that samples 3, 4 and 5 were the most susceptible to migration of its chemical constituents, followed by sample 2. In contrast, sample 1 showed the lowest migratory behaviour of its constituents. Sample 5 contains cinnamon, and the high migration rate could be attributed to this compound. However, the correlation between the essential oil type and the migration rate is not plausible for samples 1-4 because they all contain similar quantities of thyme. Thus, differences between migration rates could be attributed to other constituents found in the bioplastic composition.

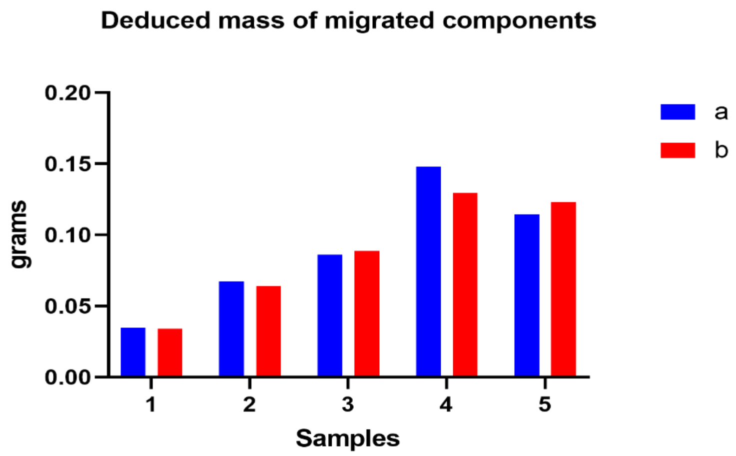

Figure 7. Migration of biologically active components from the bioplastic composition (face B) under simulated usage conditions – Test 1. *a* and *b* represent different replicates of the same sample/formulation.

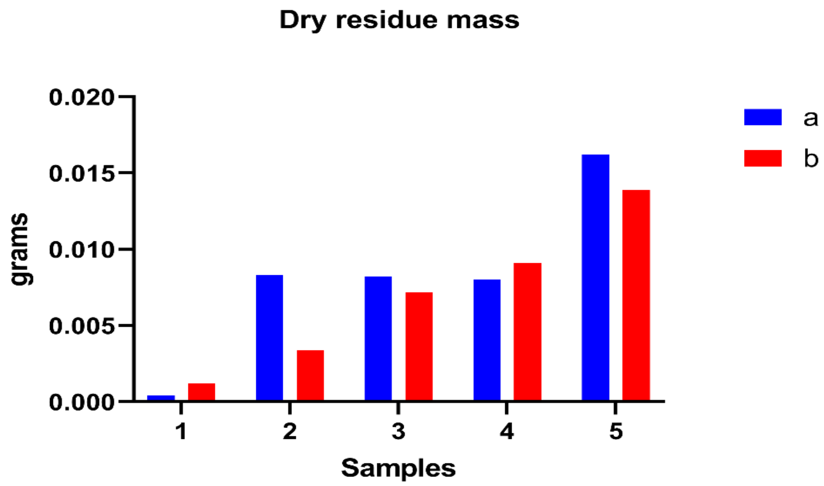

Figure 8. Migration of biologically active components from the bioplastic composition (face B) under simulated usage conditions – Test 2. *a* and *b* represent different replicates of the same sample/formulation.

The biological activity of the tested bioplastics, intended for the production of biodegradable packaging with minimal environmental impact and extended shelf life of packaged products, was highlighted through tests assessing the reduction of pathogenic microorganisms' growth and ecotoxicity. The obtained results (Table 2 and Fig. 9) demonstrated that formulation 1 inhibited the growth of *E. coli*, *S. aureus*, and *S. enterica* strains by 49-62% compared to the control, while sample 2 exhibited antimicrobial activity against *S. aureus* and *E. coli* with efficiency ranging from 58% to 61%, but did not inhibit the growth of the *S. enterica* strain. On the other hand, formulation 3 acted with an efficiency ranging from 47% to 58% against the Gram-negative strains *S. enterica* and *E. coli*, but had no effect on the Gram-positive strain *S. aureus*, while sample 4 inhibited the growth of *S. aureus*, *E. coli*, and *S. enterica* with efficiency ranging from 75% to 97%. Formulation 5 exclusively inhibited the Gram-negative strains *E. coli* and *S. aureus* by 65-91% compared to the control.

Table 2. The results of testing the influence of formulations (face B) on the growth of three bacterial strains with significance in the food industry.

Bacterial strain	Sample	CFU/mL	% reduction in bacterial growth compared to the control
S. aureus	Control (without formulation)	5.4×10^{10}	-
	Formulation 1	2.76×10^{10}	-49%
	Formulation 2	2.26×10^{10}	-58%
	Formulation 3	8.2×10^{10}	0%
	Formulation 4	1.73×10^{9}	-97%
	Formulation 5	6.2×10^{10}	0%
E. coli	Control (without formulation)	6.4×10^{9}	-
	Formulation 1	2.4×10^{9}	-62%
	Formulation 2	2.5×10^{9}	-61%
	Formulation 3	2.7×10^{9}	-58%
	Formulation 4	4.2×10^{8}	-93%
	Formulation 5	5.9×10^{8}	-91%
S. enterica	Control (without formulation)	5.7×10^{9}	-
	Formulation 1	2.2×10^{9}	-61%
	Formulation 2	6.1×10^{9}	0%
	Formulation 3	3.0×10^{9}	-47%
	Formulation 4	1.4×10^{9}	-75%
	Formulation 5	2.0×10^{9}	-65%

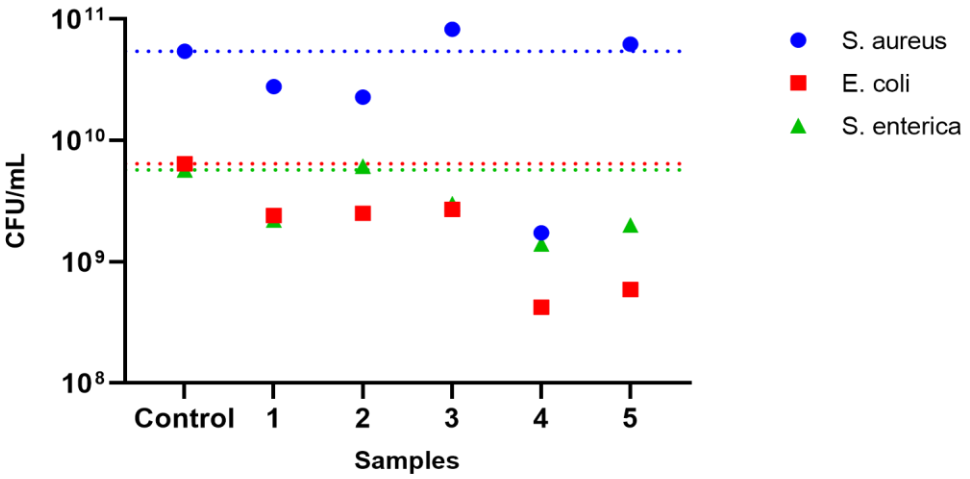

Figure 9. The effect of samples on the growth of three bacterial strains. The control was exposed to the same experimental conditions as the samples, except that it was not in contact with the recipes. The dotted lines indicate the position of the control.

Considering that all five formulations contain similar concentrations of essential oils, the significant differences in antimicrobial activity observed among samples are attributed to other physicochemical factors that favour or hinder the release of antimicrobial compounds from the polymeric matrix.

Due to the complex composition of the polymer matrix, as well as the added additives and plasticizers, some bioplastic materials obtained from the use of plant-based raw materials may, however, demonstrate potential toxicity upon degradation in the natural aquatic or terrestrial environment through the action of UV radiation or other biogeochemical factors. The products resulting from biodegradation can affect, for example, the carbon-nitrogen ratio in the soil, plant germination, or the nutrient balance in the aquatic environment (ALI et al., 2023). Ecotoxicity test results (Table 3; Fig. 10) have demonstrated that the rotifer *Brachionus calyciflorus* survival rate is not influenced by the biologically active components in the polymer matrix. In this regard, the survival rate was, in most cases 100%, except for the mixture denoted with 1, where the survival of 14 out of 15 specimens was observed.

Table 3. Ecotoxicity test results.

Formulation	No. Surviving specimens	Survival rate (%)
1	14/15	93,3%
2	15/15	100%
3	15/15	100%
4	15/15	100%
5	15/15	100%

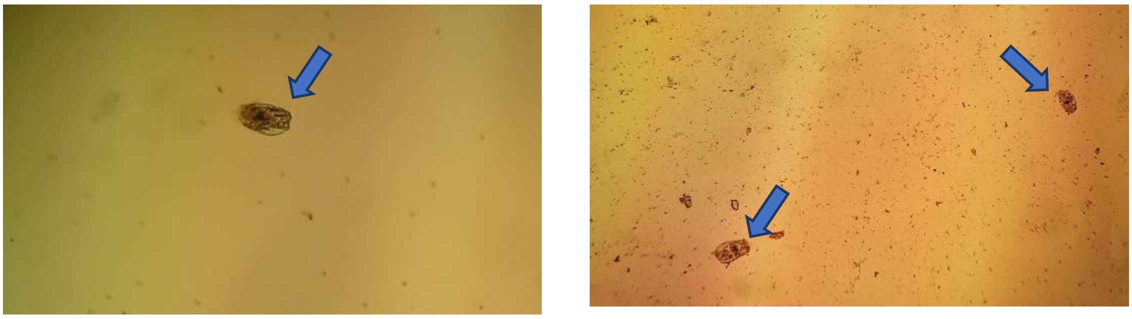

Figure 10. Viable specimens of *Brachionus* observed under the optical microscope after contact with the investigated samples.

CONCLUSIONS

The widespread use of plastic materials has led to concerning environmental issues, especially due to their rigidity and resistance to various biogeochemical factors, as well as the environmental risk posed by the chemicals present in their composition (NANNI et al., 2021). A solution to prevent these unfavourable ecological consequences may be represented by the use of bioplastic materials that are biodegradable, primarily serving as a nutrient source for the environmental microbiota, with resulting compounds having lower ecotoxicological potential (RUGINESCU et al., 2023). In this context, the results of this study have demonstrated that the investigated bioplastic materials, represented by a polymeric matrix based on polylactic acid supplemented with compounds exhibiting antimicrobial and antioxidant activity, primarily thyme essential oil and grape pomace extract, have a suitable potential for the food industry. The biopolymeric matrix exhibits a generally non-homogeneous appearance with air bubble content in some samples and the presence of insoluble particles with a reddish-brown colour, showing varying degrees of aggregation. The analysed samples present different water absorption levels ranging between 0.6% and 3.6%, depending on their predominantly non-homogeneous and variable appearance, the presence of air bubbles, and particles imparting biological properties and roughness to the bioplastic. The physical structure of the analysed bioplastic is also reflected in the migration of biologically active components from its composition under simulated conditions of use, similar to real ones, with a relatively higher migration outside the structural matrix for the formula containing cinnamon oil. The two tests used in this case, targeting the deduced mass of migrated components and the dry residue mass, have shown similar profiles of migration behaviour, but with quantitative differences.

The biological activity of the studied bioplastics was demonstrated through tests assessing the reduction in the development of pathogenic microorganisms, as well as ecotoxicity tests. The study results indicate that the tested formulations have an inhibitory effect on the growth of three bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica*. By correlating these results with those reported previously by Ruginescu et al. (2023), an increase in the shelf life of the packaged product by at least 24 to 48 hours compared to conventional packages is expected. Additionally, ecotoxicity tests have shown that the rotifer *Brachionus calyciflorus* survival rate is not influenced by the biologically active components in the polymer matrix, with the survival rate being 100% in most cases. The obtained data demonstrates that the tested bioplastic poses a very low risk to the surrounding environment, both in terrestrial and aquatic ecosystems.

Bioplastic materials are considered safe and environmentally-friendly alternatives to traditional plastics, as they are biodegradable. However, their biodegradation in natural conditions could potentially produce harmful compounds that may harm both aquatic and terrestrial environments. It is important to note that this could limit their use as a substitute for traditional plastics. Additionally, simply relying on bioplastics to solve our waste problems without changing our behaviour towards waste management is not a sustainable solution. We must take responsibility for our impact on the environment regardless of the materials we use.

AKNOWNLEDGEMENTS

This study was financially supported by S.C. MEDACRIL S.R.L. Mediaş, grant number AMBAL-INOV, My SMIS code: 120994 and the Romanian Academy project RO1567-IBB05/2023 and RO1567-IBB05/2024 granted to the Institute of Biology Bucharest.

REFERENCES

ALI S., ISHA, CHANG Y.-C. 2023. Ecotoxicological Impact of Bioplastics Biodegradation: A Comprehensive Review. *Processes*. MDPI Press. London. **11**: 3445. https://doi.org/10.3390/pr11123445 (accessed February, 2024).

ADHIKARI D., MUKAI M., KUBOTA K., KAI, T., KANEKO N., ARAKI K. S., KUBO M. 2016. Degradation of bioplastics in soil and their degradation effects on environmental microorganisms. *Journal of Agricultural Chemistry and Environment*. Academic Publisher. London. **5**: 23.

ARYAL S. 2021. Microbe Notes: McFarland Standards - Principle, Preparation, Uses, Limitations (microbenotes.com). *Basic Microbiology*. University Press. London: 15-18.

AWASTHI S. K., KUMAR M., KUMAR V., SARSAIYA S., ANERAO P., GHOSH P., SINGH L., LIU H., ZHANG Z., AWASTHI M. K. 2022. A comprehensive review on recent advancements in biodegradation and sustainable management of biopolymers. *Environmental Pollution*. Elsevier. Paris. **307**: 119600.

COJOC ROXANA, NEAGU SIMONA, DOBRE D. I., TRIFOI A., RUGINESCU R., ENACHE M. 2019. The obtaining of resveratrol from agricultural secondary flow recovery products by microbial pectinolytic extract treatment. *Rom. J. Biol. – Plant Biol*. Romanian Academy Publisher. Bucharest. **64**(1-2): 9-17.

FOLINO A., KARAGEORGIOU A., CALABR P. S., KOMILIS D. 2020. Biodegradation of wasted bioplastics in natural and industrial environments: A review. *Sustainability*. Mc Gill University Press. London. **12**: 6030.

HAN Y., TENG Y., WANG X., REN W., WANG X., LUO Y., ZHANG H., CHRISTIE P. 2021. Soil type driven change in microbial community affects poly (butylene adipate-co-terephthalate) degradation potential. *Environ. Sci. Technol*. American Chemical Society Press. New York. **55**: 4648-4657.

KALITA N. K., SARMAH A., BHASNEY S. M., KALAMDHAD A., KATIYAR V. 2021. Demonstrating an ideal compostable plastic using biodegradability kinetics of poly (lactic acid)(PLA) based green biocomposite films under aerobic composting conditions. *Environmental Challenges*. University Press. London. **3**: 100030.

KHALID M. Y., ARIF Z. U., AHMED W., ARSHAD H. 2022. Recent Trends in Recycling and Reusing Techniques of Different Plastic Polymers and Their Composite Materials. *Sustainable Materiales Technologies*. Elsevier. Paris. **31**: e00382.

KHALID M. Y., ARIF Z. U., HOSSAIN M., UMER R. 2023. Recycling of Wind Turbine Blades through Modern Recycling Technologies: A Road to ZeroWaste. *Renew. Energy Focus*. Scimago Press. London. **44**: 373-389.

MAZHANDU Z. S., MUZENDA E., MAMVURA T. A., BELAID M., NHUBU T. 2020. Integrated and consolidated review of plastic waste management and bio-based biodegradable plastics: Challenges and opportunities. *Sustainability*. Mc Gill University Press. London. **12**: 8360.

McFARLAND J. 1907. Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal Am Med Assoc*. JAMA Press. New York. **14**: 1176-1178.

MERCIU S., VACAROIU C., FILIMON R., POPESCU G., PREDA S., ANASTASESCU C., ZAHARESCU M., ENACHE M. 2009. Nanotubes biologically active in media with high salt concentration. *Biotechnol. & Biotechnol. Eq. – special issue*. Taylor & Francis Press. London. **23**(2): 827-831.

NANNI A., PARISI M., COLONNA M. 2021. Wine by-products as raw materials for the production of biopolymers and of natural reinforcing fillers: A critical review. *Polymers*. MDPI Press. London. **13**: 381.

NAVASINGH R. J. H., GURUNATHAN M. K., NIKOLOVA M. P., KRÓLCZYK J. B. 2023. Sustainable Bioplastics for Food Packaging Produced from Renewable Natural Sources. *Polymers*. MDPI Press. London. **15**: 3760. https://doi.org/10.3390/polym15183760 (accessed February, 2024).

NASROLLAHZADEH M., SHAFIEI N., NEZAFAT Z. 2021. *Application of Biopolymers in Bioplastics*. Elsevier EBooks. Amsterdam: 44 pp.

NEAGU SIMONA, PREDA S., ANASTASESCU C., ZAHARESCU M., ENACHE M., COJOC ROXANA. 2014. The functionalization of silica and titanate nanostructures with halotolerant proteases. *Revue Roumain Chimistry*. Romanian Academy Publisher. Bucharest. **59**: 97-103.

PODOSU (VLAD) AURELIA, NEAGU SIMONA, LUCACI ANCA IOANA, COJOC ROXANA, BATRÎNESCU-MOTEAU C., PURCĂREA CRISTINA, ENACHE M., RUGINESCU R. 2023. Extracellular hydrolases produced by microorganisms isolated from the polluted river Pasărea, Romania. *Romanian Journal of Biology – Plant Biology*. Romanian Academy Publisher. Bucharest. **68**(1-2): 29-40.

RUGINESCU R., BĂTRÎNESCU-MOTEAU C., LUCACI ANCA IOANA, ENACHE M., COJOC ROXANA, NEAGU SIMONA, ENACHE M. I. 2023. Microbiological characterization of raw materials and obtained biodegradable packaging. *Oltenia. Studii si Comunicari, Seria Stiintele Naturii*. Muzeul Olteniei Craiova. **39**(1): 210-220.

***. *Standard Test Method for Water Absorption of Plastics*" - ASTM D570 - 98; ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States.

Ruginescu Robert, Bătrînescu-Moteau Costin, Neagu Simona, Lucaci Anca Ioana, Cojoc Roxana, Podosu (Vlad) Aurelia, Enache Mădălin
Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest 6th, Romania.
E-mails: robert.ruginescu@ibiol.ro; costinbatrinescu@gmail.com; simona.neagu@ibiol.ro; ioanalucaci12@yahoo.com; roxana.cojoc@ibiol.ro; vladaurelia24@yahoo.com; madalin_enache@yahoo.com

Received: April 07, 2024
Accepted: July 06, 2024