PRESERVATION OF MICROMYCETES THROUGH THE LYOPHILIZATION METHOD INVOLVING NANOPARTICLES

Tamara SIRBU*, Cristina MOLDOVAN*, Olga TURCAN*

* *Institute of Microbiology and Biotechnology of Technical University of Moldova, Chisinau, Republic of Moldova*
Correspondence author: Tamara Sirbu, Institute of Microbiology and Biotechnology of Technical University of Moldova, 1 Academiei Str., MD-2028, Chişinau, Republica Moldova, phone: 0037322739609, e-mail: tamara.sirbu@imb.utm.md

Abstract. From the large number of methods used for the long-term preservation of microorganisms, the most effective are cryopreservation and lyophilization. Lyophilization is a method of preserving microorganisms, which consists of the freeze-drying process based on the removal of water from the frozen cellular material, by sublimation in a vacuum. Freezing plays an important role in the lyophilization process, as well as the lyoprotectors used. In this study, the lyoprotective medium used for lyophilization of micromycetes was supplemented with nanoparticles of Fe_2O_3, Fe_2ZnO_4, and ZnO. As a result of the evaluation of the viability of micromycetes of the genus *Aspergillus*, *Trichoderma*, and *Penicillium* after lyophilization and 1 year of storage in a lyophilized state, a stimulation of viability was obtained in the variants with Fe_2O_3 and Fe_2ZnO_4 nanoparticles in a concentration of 5.0 mg/L, and in the variants with ZnO nanoparticles after lyophilization.

Key words: micromycetes; nanoparticles; lyophilization; lyoprotective medium; viability.

INTRODUCTION

Currently, many types of nanoparticles (NPs) have been synthesized and used in various fields for various applications. There is a high interaction between NPs, cells and biomolecules. This interaction is influenced by the agglomeration, charge, chemical composition, structure, shape, size and solubility of the NPs [4]. The effectiveness of the use of NPs in various fields can be both beneficial and toxic depending on the composition, size, duration and concentration applied [2, 8, 13, 16, 29].

NPs have high activity and the ability to diffuse through biological membranes and overcome tissue barriers, so they can have a toxic effect on cells, by weakening the functions of the main components of the cell structure, such as mitochondria, nucleus, and DNA. The harmful effect of NPs on cells depends on many factors: chemical composition, concentration, exposure time etc. [6, 11, 48]. In some studies, it has been shown that exposure to Ag NPs can alter the gene expression profiles of environmental microorganisms. Lu et al. (2020), studied the effects of Ag NPs (d = 10 nm, citrate-coated) on freshwater bacteria and reported changes in gene expression, which could lead to inhibition of energy metabolism, as well as DNA replication and repair [25]. Another study indicated that microorganisms develop strategies to cope with Ag NPs toxicity. Meier et al. (2020), observed higher expression of oxidative stress response genes (e.g., superoxide dismutase, which neutralizes superoxide radicals) as well as reflux pump genes (e.g., CusA gene cation as pump which is capable of refluxing Ag^+ ions in soil microorganisms after exposure to Ag NPs (polyvinylpyrrolidone-coated) [27].

NPs with magnetic properties have the ability to respond to an external magnet, due to this fact they have found a high applicability in biomedicine, they are actively studied for the separation of biological molecules etc. [31, 35]. Currently there is a wide spectrum of magnetic NPs: based on Co, Fe, Ni, Ag, Au, Zn, iron oxides etc. The most widely used in biomedicine are Fe oxides NPs, due to their low toxicity and stability of magnetic characteristics [3, 24, 31, 34].

Iron-based magnetic nanoparticle systems have been research subjects of great interest over the last few years, both scientifically and applied, due to the numerous applications in the fields of: microelectronics, biomedicine and sensors etc. [21, 22, 36]. Due to applications in biomedicine, magnetite nanoparticles must present properties of biocompatibility, paramagnetism, specific purposes, and stability in aqueous solutions. Iron oxides have weak cytotoxicity, however, entering cells they can damage DNA and mitochondria. Therefore, in medical, biological and biotechnological applications, to avoid cell damage and death, it is recommended that iron oxide NPs be coated with a layer of biocompatible modifying component [12, 44]. The release of wastewater from various dye industries, which poses a major threat to human beings due to their hazardous health effects, is one of the pressing issues. The Fe_2ZnO_4 and Fe_2O_3 NPs are currently successfully used in the decolorization and purification of industrial wastewater, as well as in the degradation of phenol [7, 15, 17, 33]. The Fe_2O_3 NPs are used to fabricate magnetic sensing devices, in various photoelectrochemical, magneto-optical applications, in dozens of other magnetic devices and applications, such as magnetically controlled drug delivery, medical imaging, cell separation, and refrigeration [1, 30, 43]. It was also demonstrated that Fe_3O_4 and Fe_2O_3 NPs, depending on the size and applied dose, can modify the biosynthetic properties of microorganisms [40-42, 45].

The ZnO NPs are widely used in biomedicine, which have an inherent toxicity against pathogens and are used as antibacterial preparations, as well as in the treatment of cancer, diabetes mellitus, the transport of therapeutic drugs etc. [35]. The ZnO NPs are also successfully used in the pharmaceutical, food, and cosmetic industries [5, 26].

The purpose of the research was to study the influence of NPs on the viability of micromycetes preserved by the lyophilization method with the involvement of NPs.

MATERIALS AND METHODS

Twenty strains of micromycetes from the National Collection of Non-pathogenic Microorganisms from Moldova, belonging to the genera *Aspergillus*, *Trichoderma*, and *Penicillium*, were used as study objects. The lyoprotective medium for lyophilization of micromycetes was skim milk + 7.0% glucose (SM + 7.0% G). As a supplement to the lyoprotective medium SM + 7.0% G, the NPs: ZnO, Fe_2O_3 and Fe_2ZnO_4 were tested, which were selected as a result of the research carried out on the action of NPs on the growth of micromycetes [42]. Variants were mounted in which the dose of NPs supplemented in the lyoprotective medium was (mg/L): 0.1; 0.5; 1.0; 5.0; 10.0. The SM + 7.0% G variant was considered as a control (C). The size of NPs was different ZnO (20-30 nm) and were synthesized by researchers from South-West State University in Kursk, Russia [28], and NPs of Fe_2O_3 – 2-10 nm, Fe_2ZnO_4 – 8-15 nm, synthesized at the Institute of Chemistry of Moldova [14], and made available to us, to whom we thank.

Freezing was carried out in the Ultra Freezer DW86L626/386/286 refrigerator at a temperature of -50°C. The "LABCONCO 6 plus" sublimation system was used in the lyophilization process.

The rehydration of the lyophilized strains was carried out with distilled water, at a temperature of 28-30°C, for 2 hours.

Initially, the optimal concentration of NPs supplemented in the lyoprotective medium was selected.

The viability of the strains before and after lyophilization (expressed in colony-forming units – $CFU \cdot mL^{-1}$) was determined by the colony counting method on Czapek agar medium, after successive dilutions. The number of viable cells was expressed as *log*10 of CFU in 1.0 mL of suspension. Viability was calculated according to the formula $V = (log BL / log AL) \times 100$, where V is the viability of the strain in %, *log*BL – the logarithm of the CFU number before lyophilization, and *log*AL – the logarithm of the CFU number after lyophilization or storage [32].

Statistical data processing was performed using Microsoft Office Excel 2010.

RESULTS

The selection of the optimal concentration of NPs ZnO, Fe_2O_3, and Fe_2ZnO_4, supplemented in the lyoprotective medium, was carried out as a result of conducting experiences on *Penicillium* (*Talaromyces*) *funiculosum* FD 11 strain. The results obtained in these researches demonstrated that the use of ZnO NPs in low concentrations (0.1; 0.5 mg/L) has a beneficial effect on viability, and with increasing ZnO NPs concentration, viability decreases significantly. In the variants with Fe_2O_3 and Fe_2ZnO_4 NPs, in low concentrations they act as inhibitors, and in high concentrations (5.0-10.0 mg/L) they stimulate the viability of the strain. The maximum viability of the strain *P. funiculosum* FD 11 was obtained in the experimental variants with NPs of Fe_2O_3, Fe_2ZnO_4 in a concentration of 5.0 mg/L and is 88.7% and 86.0%, respectively, in comparison with the initial titer (Fig. 1).

The presented data demonstrate that ZnO NPs in a concentration of 0.1 mg/L and Fe_2O_3, Fe_2ZnO_4 NPs in concentrations 1.0, 5.0, and 10.0 mg/L are more active as lyoprotectors for protective media in the lyophilization process of micromycetes.

To confirm these statements, experiments aimed at lyophilization of the strains belonging to the *Aspergillus* and *Trichoderma* genera, used in this study, with the addition of Fe_2O_3 and Fe_2ZnO_4 NPs in the lyoprotective medium in the concentrations (mg/L): 1.0, 5.0, and 10.0. The ZnO NPs were used in a concentration of 0.1 mg/L.

Thus, the addition of Fe_2O_3 NPs (5.0 mg/L) in the lyoprotective medium, stimulates the viability of the cultures after lyophilization and exceeds the C by 6.0-14.0%, and in the variants with Fe_2ZnO_4 NPs the viability is different depending on the strain. In the *A. flavus* 3292D strain, the maximum viability after lyophilization was recorded at the concentration of Fe_2ZnO_4 NPs – 10.0 mg/L, and in the *A. alliaceus* FA 01 strain, in all tested variants, a decrease in viability was recorded in comparison with the C (Table 1).

In the strains belonging to the genus *Trichoderma*, an increased viability was also recorded in comparison with the C in the variants with NPs. As in the case of *Aspergillus* strains, the maximum value of viability was obtained in variants with NPs concentration – 5.0 mg/L. In the variants with Fe_2O_3 NPs, this value exceeds the C variant by 5.1-11.6%, and in the variants with Fe_2ZnO_4 NPs, by 8.1-16.0% (Table 2).

According to the results, the ZnO NPs in a concentration of 0.1 mg/L supplemented in the lyoprotective medium have a beneficial effect on the viability of cultures after lyophilization. Thus, the viability of *Aspergillus* strains after lyophilization increased by 4.0-12.5%, in comparison with the C variant. This stimulation was more significant in *A. fumigatus* FA 04 (12.4%) and *A. niger* FD 01 (10.6%) strains (Fig. 2).

In the strains of the genus *Trichoderma*, in the variant with ZnO NPs, viability stimulations were also recorded after lyophilization. In 4 out of 5 strains of the genus *Trichoderma*, the viability, in the variant with ZnO NPs, exceeded the C variant by 10.1-14.7%, and in the strain *T. viride* CNMN FD 17 this stimulation constituted 5.0%.

Next results obtained during the lyophilization of *Aspergillus* and *Trichoderma* strains, for the lyophilization of *Penicillium* strains, the C and the variants were supplemented with Fe_2O_3 and Fe_2ZnO_4 NPs in a concentration of 5.0 mg/L (Fig. 3).

According to the data, the viability of *Penicillium* strains, after lyophilization in the tested variants, was different (Fig. 3). In variants with NPs, viability does not vary significantly in comparison with the C variant (± 3.0%). More significant stimulation values, exceeding the C by 8.0%, were recorded in *P. viride* FD 04 and *P. viride* FD 09 strains in the variant with Fe_2ZnO_4 NPs. In some strains, insignificant decreases in viability were also recorded after lyophilization. Decreases were recorded in strains *P. funiculosum* FD 11 (- 6.5%) and *P. viride* FD 04 (- 0.8%) in the variant with Fe_2O_3 NPs, and in strains *P. verrucosum* FD 19 (-0.6%) and *P. piceum* FD 21 (-2.8%) in the variant with Fe_2ZnO_4 NPs.

Significant changes in the viability of strains of the *Penicillium* genus, after lyophilization, were not recorded even in the variant with ZnO NPs (Fig. 4).

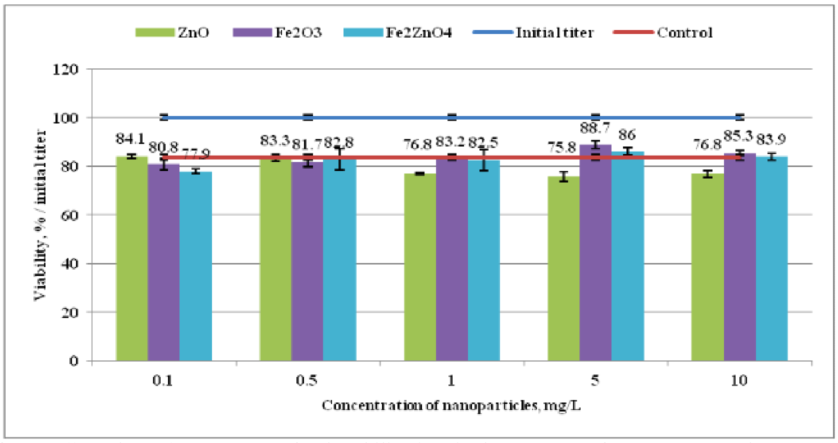

Figure 1. Viability of *Penicillium funiculosum* FD 11 after lyophilization, in the presence of ZnO, Fe_2O_3, and Fe_2ZnO_4 NPs, (% / initial titer)

Table 1. Viability of *Aspergillus* strains after lyophilization, in the presence of Fe_2O_3 and Fe_2ZnO_4 NPs supplemented in the lyoprotective medium, (% / initial titer)

Strain	Control (%)	Viability of strains after lyophilization (% / initial titer)					
		Fe_2O_3 (mg/L)			Fe_2ZnO_4 (mg/L)		
		1.0	5.0	10.0	1.0	5.0	10.0
Aspergillus flavus **3292D**	85.6±4.2	85.8±4.1	91.6±0.8	88.8±1.7	82.2±1.9	85.7±7.0	87.8±1.4
Aspergillus alliaceus **FA 01**	82.5±4.6	90.3±0.5	92.6±4.3	88.6±0.3	78.2±1.8	79.4±1.8	78.2±1.2
Aspergillus fumigatus **FA 04**	85.0±5.8	94.0±1.5	99.0±4.2	94.7±0.6	96.2±2.1	98.0±4.2	94.9±1.1
Aspergillus niger **FA 03**	77.1±3.4	83.1±0.9	85.2±3.5	82.3±2.0	82.0±1.9	85.4±0.8	83.5±1.3
Aspergillus niger **FD 01**	71.3±2.1	77.6±1.4	79.7±1.9	75.4±2.2	77.5±2.1	79.4±1.2	74.6±2.5

Note: p=0.05

Table 2. Viability of *Trichoderma* strains after lyophilization, in the presence of Fe_2O_3 and Fe_2ZnO_4 NPs supplemented in the lyoprotective medium, (% / initial titer)

Strain	Control (%)	Viability of strains after lyophilization (% / initial titer)					
		Fe_2O_3 (mg/L)			Fe_2ZnO_4 (mg/L)		
		1	5	10	1	5	10
Trichoderma virens FD 13	80.2±3.2	83.0±0.3	85.4±2.6	83.8±1.7	85.7±1.7	88.2±4.3	82.0±1.3
Trichoderma lignorum (viride) FD 14	67.0±1.3	74.4±1.6	78.6±2.7	76.2±3.1	78.8±1.6	81.5±3.5	78.5±1.2
Trichoderma koningii FD 15	66.9±2.0	66.2±1.6	68.0±3.2	67.0±1.7	74.0±1.3	76.9±1.6	75.4±2.6
Trichoderma harzianum FD16	75.5±6.2	81.2±2.4	85.0±1.8	83.8±6.0	84.1±1.6	91.5±5.7	87.4±1.6
Trichoderma viride FD 17	81.8±4.4	85.7±2.5	86.9±2.1	86.6±3.0	88.6±2.1	89.9±0.1	88.0±3.9

Note: p=0.05

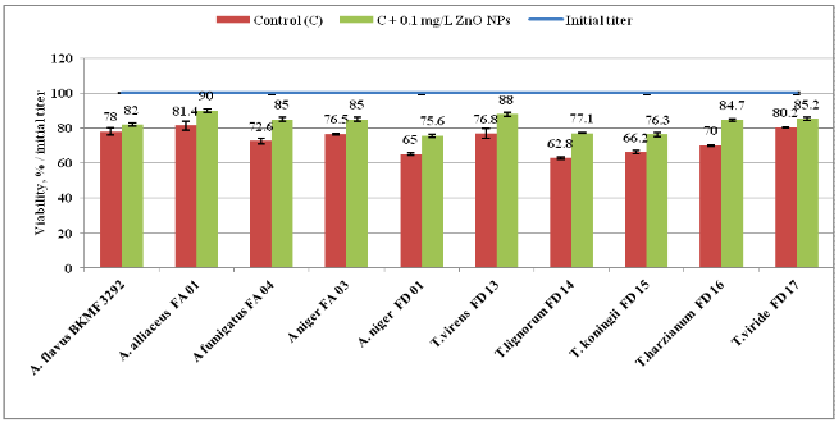

Figure 2. Viability of *Aspergillus* and *Trichoderma* strains after lyophilization, in the presence of ZnO NPs, (% / initial titer)

The viability of the ten *Penicillium* strains, after lyophilization, in the SM + 7.0% G medium supplemented with ZnO NPs varied within ± 1.0% in comparison with the C variant. In the given case ZnO NPs acted generally neutral, significant changes in the viability of the cultures after the lyophilization process were not recorded.

The evaluation of the viability of strains of micromycetes from the genus *Aspergillus*, after 1 year of preservation in a lyophilized state, demonstrated that, in the variants with Fe_2O_3 and Fe_2ZnO_4 NPs, the viability of the strains is higher, in comparison with the C variant, and in the variant with ZnO NPs is at the C level (Fig. 5). In 4 strains of *Aspergillus* in the variant with Fe_2O_3 NPs, the viability exceeded the C by 10.7-17.2%, and in 1 strain by 5.0%. The most significant results being recorded for the strain *A. fumigatus* FA 04 (117.2%) and *A. niger* FD 01 (116.3%).

In the variant with Fe_2ZnO_4 NPs, viability stimulations were also recorded in the 5 strains studied, in comparison with the C. In 4 strains, the viability varied within the limits of 112.1-121.9%, and in the strain *A. flavus* 3292D this value was 101.3%, in comparison with the C. The most significant stimulation of viability was recorded in the strain *A. fumigatus* FA 04 (121.9%).

In all studied strains from the genus *Aspergillus*, after 1 year of storage in a lyophilized state, the viability in the variant with ZnO NPs was within the limit of the C variant.

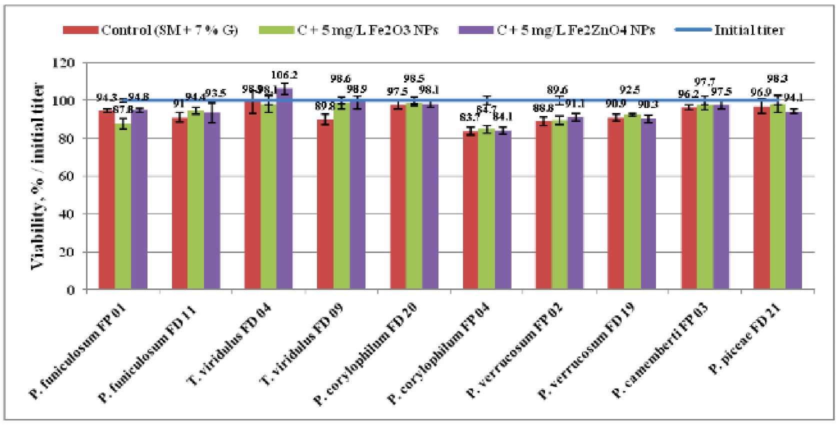

Figure 3. Viability of *Penicillium* strains after lyophilization, in the presence of Fe_2O_3 and Fe_2ZnO_4 NPs, (% / initial titer)

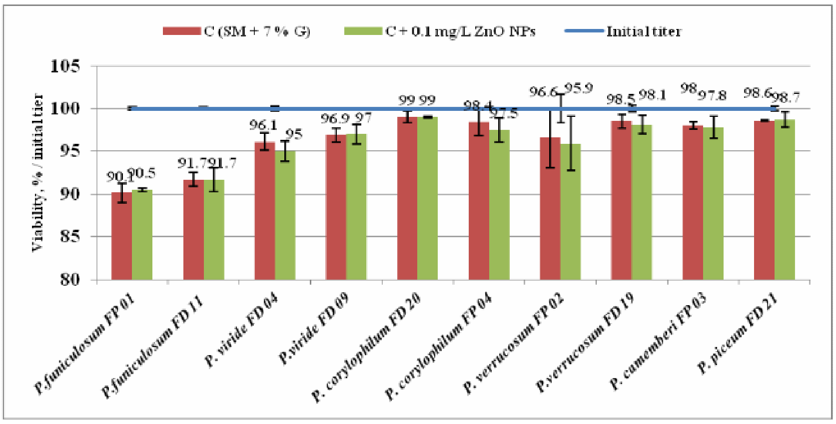

Figure 4. Viability of *Penicillium* strains after lyophilization, in the presence of ZnO NPs, (% / initial titer)

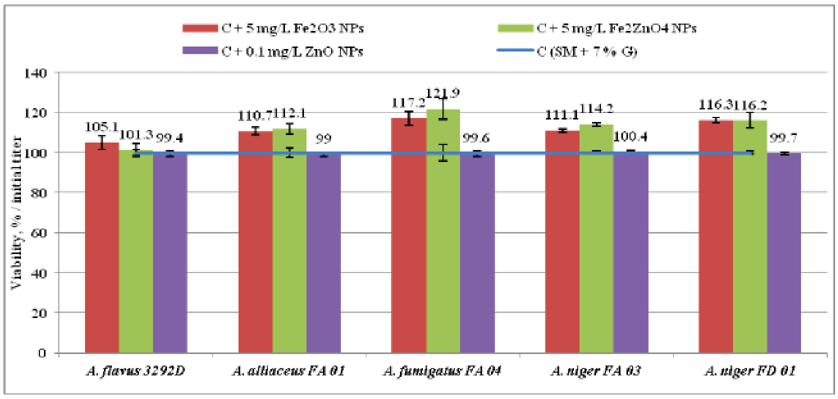

Figure 5. Viability of *Aspergillus* strains, in the presence of Fe_2O_3, Fe_2ZnO_4, and ZnO NPs, after one year of preservation in lyophilized state, (% / initial titer)

In *Trichoderma* strains, viability stimulations were also obtained in the variants with Fe_2O_3 and Fe_2ZnO_4 NPs, in comparison with the C, after 1 year of storage in a lyophilized state (Fig. 6).

The viability of *Trichoderma* strains in the variant with Fe_2O_3 NPs, after 1 year of storage in a lyophilized state, varied between 103.3-120.5%, in comparison with the C variant. Significant stimulations of viability were recorded in the strain *T. harzianum* FD 16 (120.5%) and the strain *T. lignorum* FD 14 (118.4%).

After 1 year of preservation in a lyophilized state, in the variant in which the lyoprotective medium was supplemented with Fe_2ZnO_4 NPs, the viability of *Trichoderma* strains was 106.1-122.7% in comparison with the C variant. Significant results were recorded for the strains: *T. lignorum* FD 14 (122.7%), *T. koningii* FD 15 (115.1%), and *T. harzianum* FD 16 (121.0%).

In the variant in which the lyoprotective medium was supplemented with ZnO NPs, the viability, after 1 year of preservation in a lyophilized state, did not change significantly, being within the limits of the C variant.

Penicillium strains lyophilized in lyoprotective medium supplemented with Fe_2O_3, Fe_2ZnO_4, or ZnO NPs after 1 year of preservation in a lyophilized state did not significantly change their viability, in comparison with the C variant (Fig. 7). In the variant with Fe_2O_3 NPs, the viability of *Penicillium* strains varied between 100.0-112.0%, in comparison with the C variant. The most significant stimulation of viability was recorded in the strain *P. verrucosum* FD 19 (112.0%).

In the variant in which Fe_2ZnO_4 NPs were supplemented in the lyoprotective medium, the viability of the strains, after 1 year of preservation in a lyophilized state, varied from 99.2% to 106.4%, in comparison with the C variant. A significant stimulation was recorded only in the strain *P. verrucosum* FD 19 (125.7%).

As in the case of *Aspergillus* and *Trichoderma* strains, the viability of *Penicillium* strains from the ZnO NPs variant, after 1 year of preservation in a lyophilized state, was within the limits of the C variant.

DISCUSSION

The collaboration between nanotechnologies and biotechnology can bring many new results and open new possibilities in science and technology. A number of publications highlight the toxic as well as beneficial effect of various metal nanoparticles on a specific microorganism [9, 10, 38]. Magnetic nanoparticles play an important role in biotechnology. According to

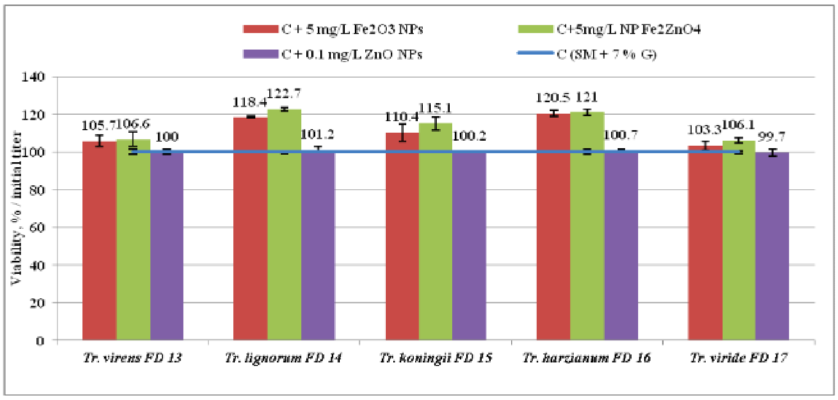

Figure 6. Viability of *Trichoderma* strains, in the presence of Fe_2O_3, Fe_2ZnO_4, and ZnO NPs, after 1 year of preservation in a lyophilized state, (% / initial titer)

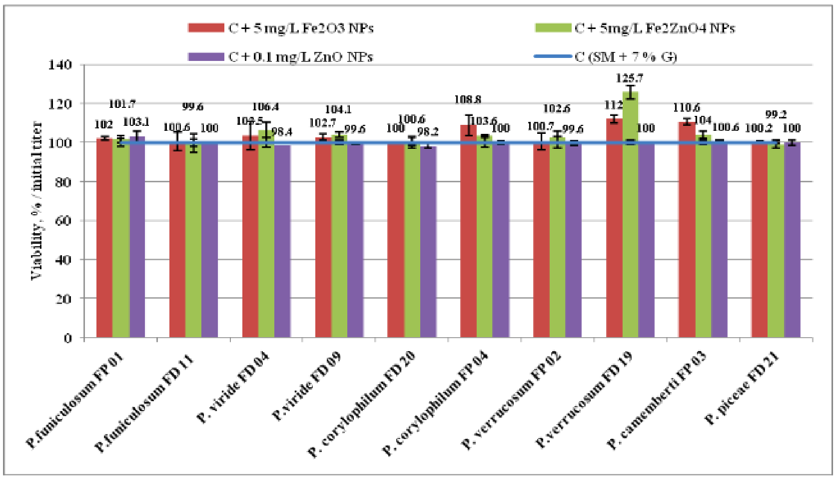

Figure 7. Viability of *Penicillium* strains, in the presence of Fe_2O_3, Fe_2ZnO_4, and ZnO NPs, after 1 year of preservation in a lyophilized state, (% / initial titer)

data from the literature, ferromagnetic nanoparticles are considered to be the support materials for the ideal immobilization of biocatalysts for easy and fast recovery [23, 36, 37].

The results obtained in this study demonstrated that: NPs of Fe_2O_3 and Fe_2ZnO_4 supplemented in the lyoprotective medium in a concentration of 5.0 mg/L can stimulate the viability of micromycete strains after lyophilization and storage in a lyophilized state. Thus, lyophilized micromycetes in the lyoprotective medium SM + 7.0% G supplemented with Fe_2O_3 NPs in a concentration of 5.0 mg/L, stimulate the viability after lyophilization of cultures of the genus *Aspergillus* by 6.0-14.0%, of the genus *Trichoderma* by 5.1-11.6%, in comparison with the C and does not significantly change the viability of cultures of the genus *Penicillium* (± 3.0% / C). After 1 year of storage in a lyophilized state, the viability of *Aspergillus* strains varied between 110.7-117.2%, *Trichoderma* strains 103.3-120.5%, and *Penicillium* strains 100-112.0% in comparison with the C.

The nanoparticles of Fe_2ZnO_4 supplemented in the lyoprotective medium SM + 7.0% G stimulate the viability after lyophilization of the cultures of the genus *Trichoderma* by 8.0-16.0%, they act neutrally on the viability of the strains of the genus *Aspergillus* and the genus *Penicillium* (± 3.0% / C). After 1 year of storage in a lyophilized state, the viability of strains from the genus *Aspergillus* varied within the limits of 112.1-121.9%, from the genus *Trichoderma* 106.1-122.7%, and for strains from the genus *Penicillium* by 99.2-106.4%, in comparison with the C variant.

We can assume that Fe_2O_3 and Fe_2ZnO_4 NPs, entering the biological liquid of the cell, come into contact with the cellular components, causing the acceleration of biosynthetic processes such as oxidative enzymes, which protect the cell from the shock produced in the lyophilization process.

The use of ZnO NPs in the lyoprotective medium SM + 7.0% G in a concentration of 0.1 mg/L has a beneficial effect on the viability after lyophilization of micromycetes, it stimulates the viability of *Aspergillus* strains by 4.0-12.5%, of *Trichoderma* strains by 10.0-14.7% in comparison with the C, and after 1 year of storage in a lyophilized state, their viability is at the level of the C variant.

The possible mechanisms of action of ZnO NPs on microorganisms, according to Jeong et al. (2020), can be: (1) morphological effect for physical deformation, (2) generation of reactive oxygen species at the sites of oxygen defects, and (3) dissolution of Zn^{2+} ions [18].

According to Jiang et al. (2020), the interaction of ZnO NPs with bacteria leads to membrane dysfunction caused by the accumulation of positively charged Zn^{2+} on the surface of the cell membrane and the disturbance of the energy metabolism of bacterial substances caused by the internalization of ZnO NPs [20].

Some studies suggest that Zn^{2+} will be electrostatically attracted to the negatively charged bacteria cell membrane surface, thereby interfering with the charge balance on the cell membrane surface, resulting in severe cell deformation, and finally leading to bacterial lysis [46]. Zhang et al. (2007), showed that ZnO NPs caused damage to the cell membrane of *E. coli*, and further research found that this damage may be caused by the direct interaction between ZnO NPs and the cell membrane [47].

The results obtained are consistent with the data presented in various scientific works according to which NPs of ZnO have the ability to quickly destroy cell membranes, having a pronounced inhibitory effect on the development of microorganisms (decrease in the diameter of colonies, and their number) [19, 20, 39]. It has also been shown that in some cases low concentrations of ZnO NPs can act beneficially on the microbial cell. The penetration of ZnO NPs into the cell directly acts on the expression of certain genes, thus directly acting on the metabolic processes in the cell [5, 7].

This could explain the positive effect of ZnO NPs on the viability of the studied cultures after lyophilization and the decrease after storage in the lyophilized state.

The use of Fe_2O_3 and Fe_2ZnO_4 NPs in the lyoprotective medium, used in the lyophilization of micromycetes, has a beneficial effect on the viability after lyophilization and storage in a lyophilized state of *Aspergillus* and *Trichoderma* strains and insignificantly on the viability of *Penicillium* strains. Also, a stimulation of the viability of the strains from the mentioned genera was obtained after lyophilization when used in the ZnO NPs lyoprotective medium, but after 1 year of storage in a lyophilized state, their viability was at the level of the C.

Significant results were obtained for *Aspergillus* strains when using lyoprotective medium SM + 7.0% G + 5.0 mg/L Fe_2O_3 NPs, which stimulates their viability after lyophilization by 6.0-14.0%, and after 1 year of preservation in a lyophilized state by 10.7-17.2%.

In the strains of micromycetes from the genus *Trichoderma*, a significant stimulation of viability was obtained when using the lyoprotective medium: SM + 7.0% G + 5.0 mg/L Fe_2ZnO_4 NPs, which stimulates their viability after lyophilization by 8.0-16.0%, in comparison with the C, and after 1 year of preservation in a lyophilized state by 6.1-22.7%.

Acknowledgements. The research was funded out within the project 20.80009.7007.09 (ANCD).

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Basavegowda, N., Mishra, K., Lee, Y.R., (2017): Synthesis, characterization, and catalytic applications of hematite (α-Fe_2O_3) nanoparticles as reusable nanocatalyst. Advances in Natural Sciences: Nanoscience and Nanotechnology, 8(2): 025017.

[2] Belova, O.B., Vinnichuk, Y.D., Berezhnaya, N.M., (2011): Functional activity of cells of the immune system of intact mice when interacting with ferromagnet nanoparticles *in vivo* and *in vitro*. (in Russian). Oncology, 13(3): 192-196.

[3] Berry, C.C., Curtis, A.S.G., (2003): Functionalisation of magnetic nanoparticles for applications in biomedicine. Journal of Physics D: Applied Physics, 36(13): R198.

[4] Bhuiyan, M.T.H., Chowdhury, M.N., Parvin, M.S., (2016): Potential nanomaterials and their applications in modern medicine: An overview. ARC Journal of Cancer Science, 2: 25-33.

[5] Bolognesi, C., Castle, L., Cravedi, J.-P., Engel, K.-H., Franz, R., Fowler, P., Grob, K., Gürtler, R., Husøy, T., Kärenlampi, S., Mennes, W., Milana, M.R., Penninks, A., Silano, V., Smith, A., Poças, M.F.T., Tlustos, C., Wölfle, D., Zorn, H., Zugravu, C.-A., (2016): Safety assessment of the substance zinc oxide, nanoparticles, for use in food contact materials. European Food Safety Authority Journal, 14(3): 4408.

[6] Chang, C., (2010): The immune effects of naturally occurring and synthetic nanoparticles. Journal of Autoimmunity, 34(3): J234-246.

[7] Chater-Sari, E., Zegadi, C., Djelloul, A., Rodane, S., Sellami, M., Kameche, M., Bettahar, N., (2020): Elaboration of copper zinc ferrite nanoparticles: Application in catalytic wet H_2O_2 oxidation of phenol. Journal of Nano- and Electronic Physics, 12(3): 03009.

[8] Chirilă, A., Doma, A.O., Cristina, R.T., (2014): Aplicații ale nanotehnologiei în biomedicină. Medicamentul Veterinar, 8(1): 12-27.

[9] Deryabina, D.G., Efremova, L.V., Karimov, I.F., Manukhov, I.V., Gnuchikh, E.Y., Miroshnikov, S.A., (2016): Comparative sensitivity of the luminescent *Photobacterium phosphoreum*, *Escherichia coli*, and *Bacillus subtilis* strains to toxic effects of carbon-based nanomaterials and metal nanoparticles. Mikrobiologiia, 85(2): 177-186.

[10] Dobias, J., (2013): Nanoparticles and microorganisms: from synthesis to toxicity. PhD thesis, Swiss Federal Institute of Technology Lausanne, Swiss Confederation.

[11] Dobrovolskaia, M.A., McNeil, S.E., (2007): Immunological properties of engineered nanomaterials. Nature nanotechnology, 2(8): 469-478.

[12] Ganapathe, L.S., Mohamed, M.A., Mohamad Yunus, R., Berhanuddin, D.D., (2020): Magnetite (Fe_3O_4) nanoparticles in biomedical application: from synthesis to surface functionalisation. Magnetochemistry, 6(4): 68.

[13] Glazko, V.I., Belopuhov, S.A., (2008): Nanotechnologies and nanomaterials in agriculture. (in Russian). Moscow: RGAU – MSHA, 228 p.

[14] Gorinchoy, V., Shova, S., Melnic, E., Kravtsov V., Turta, C., (2013): Homotrinuclear Fe3III μ – oxo salicylate cluster. Synthesis, structure and properties. Chemistry Journal of Moldova, 8(2): 83-89.

[15] Grover, V.A., Hu, J., Engates, K.E., Shipley, H.J., (2012): Adsorption and desorption of bivalent metals to hematite nanoparticles. Environmental Toxicology and Chemistry, 31(1): 86-92.

[16] Ingle, A.P., Duran, N., Rai, M., (2014): Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review. Applied Microbiology and Biotechnology, 98(3): 1001-1009.

[17] Jeon, B.H., Dempsey, B.A., Burgos, W.D., Royer, R.A., (2003): Sorption kinetics of Fe(II), Zn(II), Co(II), Ni(II), Cd(II), and Fe(II)/Me(II) onto hematite. Water Research, 37(17): 4135-4142.

[18] Jeong, E., Kim, C.U., Byun, J., Lee, J., Kim, H.-E., Kim, E.-J., Choi, K.J., Hong, S.W., (2020): Quantitative evaluation of the antibacterial factors of ZnO nanorod arrays under dark conditions: physical and chemical effects on *Escherichia coil* inactivation. The Science of the Total Environment, 712: 136574.

[19] Jiang J., Pi, J., Cai, J., (2018): The advancing of zinc oxide nanoparticles for biomedical applications. Bioinorganic Chemistry and Application, 2018: 1062562.

[20] Jiang, S., Lin, K., Cai, M., (2020): ZnO nanomaterials: Current advancements in antibacterial mechanisms and applications. Frontiers in Chemistry, 8: 580.

[21] Kashuba, N.A., (2020): On approaches to assessing the effect of nanoparticles on the human body. (in Russian). Hygiene and sanitation, 99(5): 443-447.

[22] Kita, E., Oda, T., Kayano, T., Sato, S., Minagawa, M., Yanagihara, H., Kishimoto, M., Mitsumata, C., Hashimoto, S., Yamada, K., Ohkohchi, N., (2010): Ferromagnetic nanoparticles for magnetic hyperthermia and thermoablation therapy. Journal of Physics D Applied Physics, 43(47): 1-9.

[23] Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., Muller, R.N., (2008): Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chemical Reviews, 108(6): 2064-2110.

[24] Lu, A.H., Salabas, E.L., Schüth, F., (2007): Magnetic nanoparticles: synthesis, protection, functionalization, and application. Angewandte Chemie (International Edition in English), 46(8): 1222-1244.

[25] Lu, T., Qu, Q., Lavoie, M., Pan, X., Peijnenburg, W.J.G.M., Zhou, Z., Pan, X., Cai, Z., Qian, H., (2020): Insights into the transcriptional responses of a microbial community to silver nanoparticles in a freshwater microcosm. Environmental Pollution (Barking, Essex : 1987), 258: 113727.

[26] Malhotra, S.P.K., Mandal, T.K., (2019): Zinc oxide nanostructure and its application as agricultural and industrial material. Contaminants in Agriculture and Environment: Health Risks and Remediation, 1: 217-220.

[27] Meier, M.J., Dodge, A.E., Samarajeewa, A.D., Beaudette, L.A., (2020): Soil exposed to silver nanoparticles reveals significant changes in community structure and altered microbial transcriptional profiles. Environmental Pollution (Barking, Essex : 1987), 258: 113816.

[28] Mirgorod, Y.A., Borodina, V.G., (2013): Preparation and bactericidal properties of silver nanoparticles in aqueous tea leaf extract. Inorganic Materials, 49(10): 980-983.

[29] Mody, V.V., Nounou, M.I., Bikram, M., (2009): Novel nanomedicine-based MRI contrast agents for gynecological malignancies. Advanced Drug Delivery Reviews, 61(10): 795-807.

[30] Mohapatra, M., Anand, S., (2010): Synthesis and applications of nano-structured iron oxides/hydroxides – a review. International Journal of Engineering, Science and Technology, 2(8): 127-146.

[31] Mornet, S., Vasseur, S., Grasset, F., Veverka, P., Goglio, G., Demourgues, A., Portier, J., Pollert, E., Duguet, E., (2006): Magnetic nanoparticle design for medical applications. Progress in Solid State Chemistry, 34(2-4): 237-247.

[32] Muñoz-Rojas, J., Bernal, P., Duque, E., Godoy, P., Segura, A., Ramos, J.L., (2006): Involvement of cyclopropane fatty acids in the response of *Pseudomonas putida* KT2440 to freeze-drying. Applied and Environmental Microbiology, 72(1): 472-477.

[33] Nawaz, A., Khan, A., Ali, N., Mao, P., Gao, X., Ali, N., Bilal, M., Khan, H., (2022): Synthesis of ternary-based visible light nano-photocatalyst for decontamination of organic dyes-loaded wastewater. Chemosphere, 289: 133121.

[34] Pershina, A.G., Sazonov, A.E., Milto, I.V., (2008): Use of magnetic nanoparticles in biomedicine. (in Russian). Bulletin of Siberian Medicine, 2: 70-78.

[35] Purushotham, S., Ramanujan, R., (2010): Modeling the performance of magnetic nanoparticles in multimodal cancer therapy. Journal of Applied Physics, 107(11): 114701-114709.

[36] Rashid, S.S., Mustafa, A.H., Ab Rahim, M.H., (2022): Ferromagnetic nanoparticles synthesis and functionalization for laccase enzyme immobilization. Materialstoday: Proccedings, 48(4): 916-919.

[37] Safarik, I., Safarikova, M., (2009): Magnetic nano- and microparticles in biotechnology. Chemical Papers, 63(5): 497-505.

[38] Shahzeidi, Z.S., Amiri, G., (2015): Antibacterial activity of Fe_3O_4 nanoparticles. International Journal of Bio-Inorganic Hybrid Nanomaterials, 4(3): 135-140.

[39] Shcherbakov, A.B., Ivanov, V.K., (2019): Workshop on nanomaterials and nanotechnology. (in Russian). Moscow: Moscow University Press, 368 p.

[40] Shelest, N.A., Volkova, E.K., Kozina, K.V., Korchenova, M.V., Tuchina, E.S., Zakharevich, A.M., Kochubey, V.I., Tuchin, V.V., (2014): Effect of blue LED (405 nm) radiation and iron (III) oxide nanoparticles on the survival and morphology of *Staphylococcus aureus* 209 P cells. (in Russian). News of the Saratov University. New series. Chemistry. Biology. Ecology, 14(4): 62-68.

[41] Sîrbu, T., Timuş, I., (2018): Efectul nanoparticulelor de Fe_2O_3, Fe_2ZnO_4 şi Fe_2CuO_4 asupra activităţii biosintetice a micromicetelor. pp. 371-374. In Sîrbu, T., Timuş, I., Gorincioi, V., Maslobrod, S., (eds.): International Scientific Conference – Plant Protection in Conventional and Ecological Agriculture. Chișinău: Biotehdesign.

[42] Sîrbu, T.F., Timuş, I.N., (2019): Growth of micromycetes in the presence of nanoparticles. (in Russian). pp. 50-52. In Sîrbu, T.F., Timuş, I.N., Gorincioi, V.V., Moldovan, C.E., Ţurcan, O.P., (eds.): Biologically active preparations for plant growing proceeding. XV International scientific-applied conference, Kyiv.

[43] Son, S., Jung, P.-H., Park, J., Huh, D., Kim, K., Lee, H.-C., Lee, H., (2018): Nano- and micro-sized Fe_2O_3 structures fabricated by UV imprint lithography. Special Issue: Advanced Electromaterials, 215(20): 1700948.

[44] Turanskaya, S.P., Kusyak, A.P., Turov, V.V., Gorbik, P.P., (2013): Interaction of magnetic nanoparticles with cells. (in Russian). Surface, 20(5): 227-246.

[45] Usatîi, A., Chiseliţa, N., (2018): Profilul activităţii catalazei şi producerii de proteine la *Saccharomyces cerevisiae* CNMN-Y-20 sub influenţa nanoparticulelor ZnO. Studia Universitatis Moldaviae, Seria "Ştiinţe reale și ale naturii", 111(1): 92-96.

[46] Wang, Y.-W., Cao, A., Jiang, Y., Zhang, X., Liu, J.-H., Liu, Y., Wang, H., (2014): Superior antibacterial activity of ZnO/graphene oxide composites originated from high zinc concentration localized around bacteria. ACS Applied Materials and Interfaces, 6: 2791-2798.

[47] Zhang, L., Jiang, Y., Ding, Y., Povey, M., York, D., (2007): Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). Journal of Nanoparticle Research volume, 9: 479-489.

[48] Zolnik, B.S., González-Fernández, A., Sadrieh, N., Dobrovolskaia, M.A., (2010): Nanoparticles and the immune system. Endocrinology, 151(2): 458-465.

Received: April 6, 2023
Accepted: May 31, 2023
Published Online: June 5, 2023
Analele Universității din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House