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 \( \text{Fe}_2\text{ZnO}_4 \) and \( \text{Fe}_2\text{O}_3 \) 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 \( \text{Fe}_2\text{O}_3 \) 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 \( \text{Fe}_2\text{O}_3 \) and \( \text{Fe}_2\text{ZnO}_4 \) 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, Fe₂O₃, and Fe₂ZnO₄ 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 Fe₂O₃ – 2-10 nm, Fe₂ZnO₄ – 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∙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, Fe₂O₃, and Fe₂ZnO₄, 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 Fe₂O₃ and Fe₂ZnO₄ 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 Fe₂O₃, Fe₂ZnO₄ 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 Fe₂O₃, Fe₂ZnO₄ 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 Fe₂O₃ and Fe₂ZnO₄ 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 Fe₂O₃ 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 Fe₂ZnO₄ 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 Fe₂ZnO₄ 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 Fe₂O₃ NPs, this value exceeds the C variant by 5.1-11.6%, and in the variants with Fe₂ZnO₄ 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 Fe₂O₃ and Fe₂ZnO₄ 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$_2$ZnO$_4$ 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$_2$O$_3$ NPs, and in strains *P. verrucosum* FD 19 (- 0.6%) and *P. piceum* FD 21 (- 2.8%) in the variant with Fe$_2$ZnO$_4$ 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$_2$O$_3$, and Fe$_2$ZnO$_4$ NPs, (% / initial titer)

**Table 1.** Viability of *Aspergillus* strains after lyophilization, in the presence of Fe$_2$O$_3$ and Fe$_2$ZnO$_4$ NPs supplemented in the lyoprotective medium, (% / initial titer)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control (%)</th>
<th>Fe$_2$O$_3$ (mg/L)</th>
<th>1.0</th>
<th>5.0</th>
<th>10.0</th>
<th>Fe$_2$ZnO$_4$ (mg/L)</th>
<th>1.0</th>
<th>5.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus flavus</em> 3292D</td>
<td>83.6±4.2</td>
<td>85.6±4.1</td>
<td>91.6±0.8</td>
<td>88.8±1.7</td>
<td>82.2±1.9</td>
<td>85.7±1.9</td>
<td>87.8±1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus alliaceus</em> FA 01</td>
<td>82.5±4.6</td>
<td>90.3±0.5</td>
<td>92.6±4.3</td>
<td>88.6±0.3</td>
<td>78.2±1.8</td>
<td>79.4±1.8</td>
<td>78.2±1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em> FA 04</td>
<td>85.0±5.8</td>
<td>94.0±1.5</td>
<td>99.0±4.2</td>
<td>94.7±0.6</td>
<td>96.2±2.1</td>
<td>98.0±4.2</td>
<td>94.9±1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em> FA 03</td>
<td>77.1±3.4</td>
<td>83.1±0.9</td>
<td>85.2±3.5</td>
<td>82.3±2.0</td>
<td>82.0±1.9</td>
<td>85.4±0.8</td>
<td>83.5±1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em> FD 01</td>
<td>71.3±2.1</td>
<td>77.6±1.4</td>
<td>79.7±1.9</td>
<td>75.4±2.2</td>
<td>77.5±2.1</td>
<td>79.4±1.2</td>
<td>74.6±2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: p<0.05

**Table 2.** Viability of *Trichoderma* strains after lyophilization, in the presence of Fe$_2$O$_3$ and Fe$_2$ZnO$_4$ NPs supplemented in the lyoprotective medium, (% / initial titer)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control (%)</th>
<th>Fe$_2$O$_3$ (mg/L)</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>Fe$_2$ZnO$_4$ (mg/L)</th>
<th>1</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichoderma virens</em> FD 13</td>
<td>80.2±3.2</td>
<td>83.0±0.3</td>
<td>83.4±2.6</td>
<td>83.8±1.7</td>
<td>85.7±1.7</td>
<td>88.2±4.3</td>
<td>82.0±1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma lignorum</em> (viride) FD 14</td>
<td>67.0±1.3</td>
<td>74.4±1.6</td>
<td>78.6±2.7</td>
<td>76.2±3.1</td>
<td>78.8±1.6</td>
<td>81.5±3.5</td>
<td>78.5±1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma koningii</em> FD 15</td>
<td>66.9±2.0</td>
<td>66.2±1.6</td>
<td>68.0±3.2</td>
<td>67.0±1.7</td>
<td>74.0±1.3</td>
<td>76.9±1.6</td>
<td>75.4±2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma harzianum</em> FD16</td>
<td>75.5±6.2</td>
<td>81.2±2.4</td>
<td>85.0±1.8</td>
<td>83.8±6.0</td>
<td>84.1±1.6</td>
<td>91.5±5.7</td>
<td>87.4±1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma viride</em> FD 17</td>
<td>81.8±4.4</td>
<td>85.7±2.5</td>
<td>86.9±2.1</td>
<td>86.6±3.0</td>
<td>88.6±2.1</td>
<td>89.9±0.1</td>
<td>88.0±3.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 Fe₂O₃ and Fe₂ZnO₄ NPs, the viability of the strains is higher, in comparison with the C variant. In 4 strains of *Aspergillus* in the variant with Fe₂O₃ NPs, 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 *Trichoderma* strains, viability stimulations were also obtained in the variants with Fe$_2$O$_3$ and Fe$_2$ZnO$_4$ 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$_2$O$_3$ 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$_2$ZnO$_4$ NPs, the viability of *Trichoderma* strains was 106.1-122.7% in comparison with the C variant. Significant stimulations of viability were also obtained in the variants with Fe$_2$O$_3$ NPs, after 1 year of preservation in a lyophilized state (Fig. 7). In the variant in which *T. lignorum* FD 14 (118.4%) was supplemented with ZnO NPs, the viability, 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 *P. verrucosum* FD 19 (121.0%). In the variant in which *Fe$_2$ZnO$_4$* 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...
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 Fe₂O₃ and Fe₂ZnO₄ 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 Fe₂O₃ 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 Fe₂ZnO₄ 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 Fe₂O₃ and Fe₂ZnO₄ 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 Zn²⁺ 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 Zn²⁺ 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 Zn²⁺ 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 Fe₂O₃ and Fe₂ZnO₄ 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 Fe₂O₃ 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 Fe₂ZnO₄ 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%.

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

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