

## THE INFLUENCE OF SILVER NANOPARTICLES ON GAMETOPHYTE DIFFERENTIATION IN SOME LEPTOSPORANGIATE FERNS

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### Abstract

Currently, silver nanoparticles (AgNPs) have a wide applicability and are being used in more and more fields, including industrial, medical, agricultural, environmental protection, etc. Once in the environment, nanoparticles can have negative effects on living things or neutral or beneficial effects. The aim of this research was to highlight the influence of silver nanoparticles on spore germination and gametophyte differentiation in *Asplenium scolopendrium*, *Athyrium filix-femina*, and *Dryopteris filix-mas* fern species. In the *in vitro* experiment, AgNPs (Thermo Scientific Chemicals) with a size of 20 nm, 0.02mg/mL, supplied in 2mM sodium citrate, diluted 10 (N1), 100 (N2), and 1000x (N3) were used. The control variants were cultivated on Knop solution (Control 1), as well as on the mixture of Knop solution and 2mM sodium citrate (1:1) (Control 2). The cultures maintained at room temperature (18±2°C) were periodically analyzed microscopically after 3 and 8 weeks, respectively. The results obtained indicate that the AgNPs solution, dilution 10<sup>-1</sup>, inhibits spore germination in the three tested species. The other two nanoparticle solutions (dilutions 10<sup>-2</sup> and 10<sup>-3</sup>) to which the spores and gametophytes were exposed determined a gametophyte differentiation similar to the control variant.

**Keywords:** spores, ferns, silver nanoparticles, phytotoxicity.

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### 1. INTRODUCTION

From the invention of nanotechnology in the 1980s, many applications with nanomaterials were developed in various sectors (Almatroudi, 2020). A subset of nanomaterials are nanoparticles, which have a three-dimensional nanoscale size and generally have spherical or quasi-spherical shapes (Rodrigues et al., 2024). Metallic nanoparticles are extensively studied nowadays because of their unique electronic, magnetic, chemical, and mechanical properties (Asif et al., 2022).

Silver nanoparticles (AgNPs) are an important innovation in nanotechnology (Dhaka et al., 2023) and they have a wide applicability due to their attractive physicochemical properties and biological functionality, being used in more and more fields, including industrial, medical, agricultural, environmental protection, etc.

Once in the environment, nanoparticles can have negative, neutral, or beneficial effects on living things. Although AgNPs toxicity is examined along with possible toxicity pathways, standardized protocols for comprehensive toxicity assessment are still lacking (Hosny et al., 2025).

Even though the biological toxicity mechanism of AgNPs has been debated for decades, there is an increasing number of studies on various organisms, including bacteria, algae, invertebrates, and higher plants.

Ferns, known as cryptogams, are the most primitive group of vascular plants that make up more than 90% of the extant diversity (Chen et al., 2023) and consist of 13,600 species and 400 genera (Nagajyothi et al., 2024).

Therefore, the inclusion of this group in standard ecotoxicological tests is an important objective in terms of ecological relevance. Testing species representative of the studied ecosystem would contribute considerably to the interpretation and extrapolation of laboratory results.

Pteridophytes are divided based on sporangial development into: eusporangiate (large sporangia developed from many initial cells and producing hundreds of spores) and leptosporangiate (small sporangia developed from a single cell and producing a small number of spores) groups (Nowak et al., 2022). Leptosporangiate are known from the Carboniferous period to the present, including a great number of fossil and living species (Frojdová et al., 2021).

Spores are haploid cells from which a small gametophyte develops. The gametophyte has long been recognized as a useful model in plant research. The small size and the possibility of culturing the gametophyte in aqueous suspension substantially simplify laboratory procedures and reduce the costs of standard assays while maintaining the biological relevance of whole plant testing.

The aim of this research was to highlight the influence of silver nanoparticles on spore germination and gametophyte differentiation in *Asplenium scolopendrium* L., *Athyrium filix-femina* (L.) Roth, and *Dryopteris filix-mas* (L.) Schott fern species.

## 2. MATERIALS AND METHODS

Mature leaves were collected from Vâlsan Valley from several individuals belonging to the three fern species. They were transported in the laboratory, placed face down on sheets of paper, and kept at room temperature to release the spores from the sporangia. The spores were collected and stored by dry method (Soare and Aldoiu, 2010).

In the *in vitro* experiment, AgNPs (Thermo Scientific Chemicals) with a size of 20 nm, 0.02mg/mL, supplied in 2mM sodium citrate, diluted 10 (N1), 100 (N2), and 1000x (N3) were used. The control variants were cultivated on Knop solution (Control 1), as well as on the mixture of Knop solution and 2mM sodium citrate (1:1) (Control 2) (Table 1). The cultures maintained at room temperature ( $18\pm 2^{\circ}\text{C}$ ) were periodically analyzed microscopically after 3 and 8 weeks, respectively.

The biological material was microphotographed using a digital optical microscope Eurotek Orma E5.

Table 1. Tested variants

| Variant      | Contain                                    | Dilution | Concentration         |
|--------------|--------------------------------------------|----------|-----------------------|
| C1 (Control) | Knop Solution                              | -        | -                     |
| C2           | Knop solution and 2mM sodium citrate (1:1) | -        | -                     |
| N1           | AgNPs in 2mM sodium citrate solution       | 10       | 2 $\mu\text{g/mL}$    |
| N2           |                                            | 100      | 0.2 $\mu\text{g/mL}$  |
| N3           |                                            | 1000     | 0.02 $\mu\text{g/mL}$ |

## 3. RESULTS AND DISCUSSIONS

The spores are considered germinated when the initial cell of the rhizoid is visible. In addition to germinated spores, the main stages of development observed during the test period are: prothalian

filaments (variable number of cells with chloroplasts), lamella (the longitudinal division of the end cell of the prothalian filament begins), and differentiation of antheridia with viable antherozoids.

After three weeks of exposure to variants C1 and C2, spore germination and filament formation were observed across all species, with C2 often leading to more advanced structures like surface films or branched filaments (Fig. 1-2, 6-7, 11-12).

The N1 variant inhibited spore germination, partially or completely (*Athyrium filix-femina*), while the N2 and N3 variants supported the growth of prothallian filaments (*Asplenium scolopendrium*), with varying degrees of branching and cell elongation (*Athyrium filix-femina*). *Dryopteris filix-mas* exhibits the most consistent germination and filament development across all conditions, though with some deformities in N2 and N3 (Table 2)(Fig. 3-5, 8-10, 13-15).

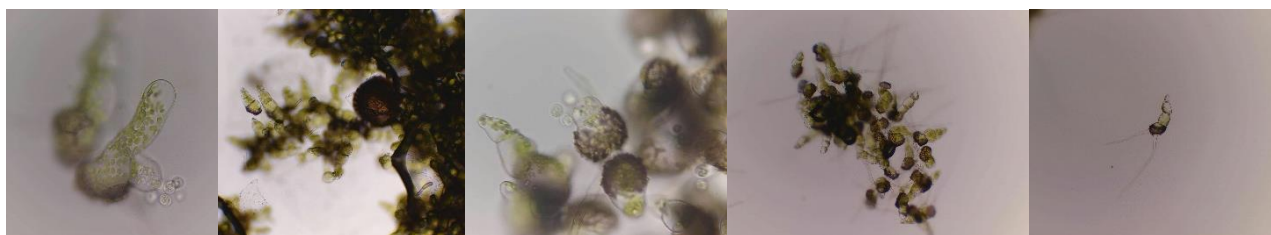
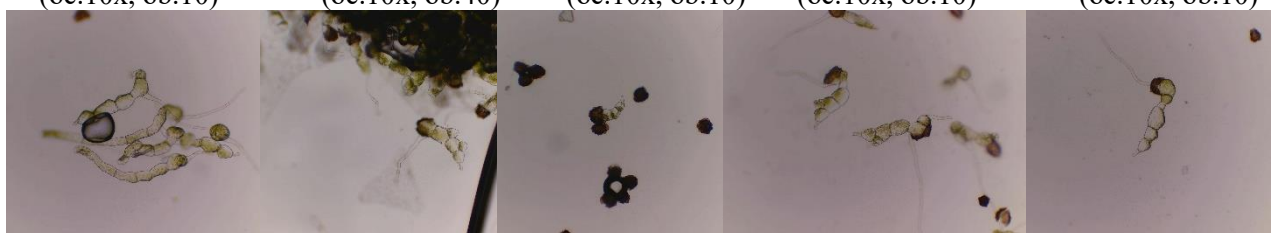
After 8 weeks of exposure, the observations indicate significant variations in prothallus development depending on the species and treatment conditions (C1, C2, N1, N2, and N3)(Fig. 16-30).

The highest inhibition of spores germination was registered in variant N1 in *Athyrium filix-femina* and *Dryopteris filix-mas*, whereas in *Asplenium scolopendrium* fern, short filaments predominated. Even though there were observed deformed cells in N2 in *Asplenium scolopendrium*, with the decreasing of nanoparticle concentration the development of prothalian filaments and lamella formation are allowed in all fern species.

Overall, control conditions tend to support normal development, while N1, N2, and N3 treatments diversely affect spore germination and morphology (Table 3).

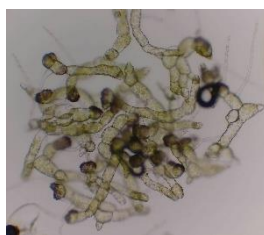
**Table 2. Gametophyte differentiation after 3 weeks of exposure**

| Control (C1)                                                                                        | C2                                                                                                                                                                                                                 | N1                                                                                                     | N2                                                                                                                                           | N3                                                                                                  |
|-----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| <b><i>Asplenium scolopendrium (As)</i></b>                                                          |                                                                                                                                                                                                                    |                                                                                                        |                                                                                                                                              |                                                                                                     |
| Prothalian filaments, some branched. Antheridia on filaments, viable antherozoids - <b>Figure 1</b> | Prothalian filaments with shorter cells. Surface film on medium - <b>Figure 2</b>                                                                                                                                  | Germinated spores, very short filaments, basal cell with antheridia - <b>Figure 3</b>                  | Prothalian filaments - <b>Figure 4</b>                                                                                                       | Prothalian filaments - <b>Figure 5</b>                                                              |
| <b><i>Athyrium filix-femina (Aff)</i></b>                                                           |                                                                                                                                                                                                                    |                                                                                                        |                                                                                                                                              |                                                                                                     |
| Germinated spores and prothalian filaments - <b>Figure 6</b>                                        | Filament with 2–3 cells and germinated spores; rhizoids may be surrounded by crystalline deposits and may detach from the cell from which they formed. A film forms on the surface of the medium - <b>Figure 7</b> | Spores not germinated - <b>Figure 8</b>                                                                | Prothalian filaments with 3–4 cells and the beginning stage of prothallian lamella formation - <b>Figure 9</b>                               | Prothalian filaments with 5 cells, some cells elongated, some filaments branched - <b>Figure 10</b> |
| <b><i>Dryopteris filix-mas (Dfm)</i></b>                                                            |                                                                                                                                                                                                                    |                                                                                                        |                                                                                                                                              |                                                                                                     |
| Germinated spores and prothalian filaments with 4–6 cells, sometimes 8 cells - <b>Figure 11</b>     | Branched filaments - <b>Figure 12</b>                                                                                                                                                                              | Germinated spores (10%) and filaments with 2–4 cells, no rhizoids or short rhizoids - <b>Figure 13</b> | Branched prothalian filaments with deformed cells, with rhizoids and the beginning stage of prothallian lamella formation - <b>Figure 14</b> | Prothalian filaments with 2–6 cells; some cells are constricted = strangled - <b>Figure 15</b>      |

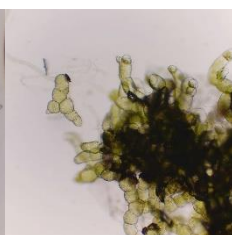
**Figure 1.** As-C1  
(oc.10x, ob.40)**Figure 2.** As-C2  
(oc.10x, ob.10)**Figure 3.** As-N1  
(oc.10x, ob.40)**Figure 4.** As-N2  
(oc.10x, ob.10)**Figure 5.** As-N3  
(oc.10x, ob.10)**Figure 6.** Aff-C1  
(oc.10x, ob.10)**Figure 7.** Aff-C2  
(oc.10x, ob.40)**Figure 8.** Aff-N1  
(oc.10x, ob.10)**Figure 9.** Aff-N2  
(oc.10x, ob.10)**Figure 10.** Aff-N3  
(oc.10x, ob.10)**Figure 11.** Dfm-C1  
(oc.10x, ob.10)**Figure 12.** Dfm-C2  
(oc.10x, ob.10)**Figure 13.** Dfm-N1  
(oc.10x, ob.10)**Figure 14.** Dfm-N2  
(oc.10x, ob.10)**Figure 15.** Dfm-N3  
(oc.10x, ob.10)**Table 3. Gametophyte differentiation after 8 weeks of exposure**

| Control (C1)                                                                               | C2                                                                             | N1                                                                                                        | N2                                                                                                  | N3                                                                         |
|--------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| <b><i>Asplenium scolopendrium</i></b>                                                      |                                                                                |                                                                                                           |                                                                                                     |                                                                            |
| Prothalian filaments and the stage of lamella formation - <b>Figure 16</b>                 | Prothalian filaments and the stage of lamella formation - <b>Figure 17</b>     | Very short filaments with antheridia, very rarely the stage of lamella formation - <b>Figure 18</b>       | Prothalian filaments, some branched, deformed cells - <b>Figure 19</b>                              | Prothalian filaments - <b>Figure 20</b>                                    |
| <b><i>Athyrium filix-femina</i></b>                                                        |                                                                                |                                                                                                           |                                                                                                     |                                                                            |
| Prothalian filaments with 9–10 cells and the stage of lamella formation - <b>Figure 21</b> | Short, damaged filaments and ungerminated spores (majority) - <b>Figure 22</b> | Ungerminated spores, a few filaments with 2–3 cells, and very rarely germinated spores - <b>Figure 23</b> | Prothalian filaments branched from the base and prothalian lamella formation - <b>Figure 24</b>     | Prothalian filaments with very long cells - <b>Figure 25</b>               |
| <b><i>Dryopteris filix-mas</i></b>                                                         |                                                                                |                                                                                                           |                                                                                                     |                                                                            |
| Prothalian filaments and lamellas - <b>Figure 26</b>                                       | Short prothalian filaments with swollen cells - <b>Figure 27</b>               | Germinated spores (10%) and ungerminated spores - <b>Figure 28</b>                                        | Long prothallial filaments, sometimes branched, and prothalian lamella formation - <b>Figure 29</b> | Prothalian filaments and the stage of lamella formation - <b>Figure 30</b> |

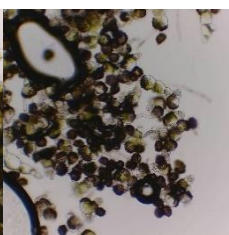




**Figure 16.** As-C1  
(oc.10x, ob.10)



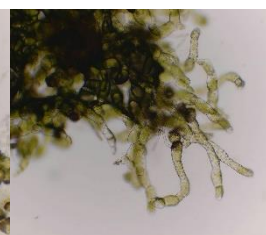
**Figure 17.** As-C2  
(oc.10x, ob.40)



**Figure 18.** As-N1  
(oc.10x, ob.10)



**Figure 19.** As-N2  
(oc.10x, ob.10)



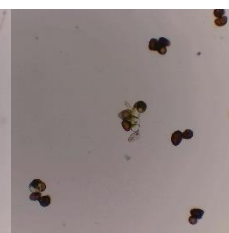
**Figure 20.** As-N3  
(oc.10x, ob.10)



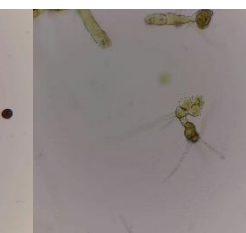
**Figure 21.** Aff-C1  
(oc.10x, ob.10)



**Figure 22.** Aff-C2  
(oc.10x, ob.40)



**Figure 23.** Aff-N1  
(oc.10x, ob.10)



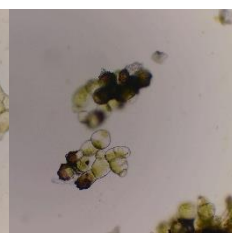
**Figure 24.** Aff-N2  
(oc.10x, ob.10)



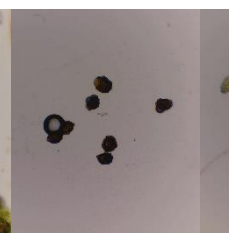
**Figure 25.** Aff-N3  
(oc.10x, ob.10)



**Figure 26.** Dfm-C1  
(oc.10x, ob.10)



**Figure 27.** Dfm-C2  
(oc.10x, ob.40)



**Figure 28.** Dfm-N1  
(oc.10x, ob.10)



**Figure 29.** Dfm-N2  
(oc.10x, ob.10)



**Figure 30.** Dfm-N3  
(oc.10x, ob.10)

Due to their antibacterial, antifungal, antiviral, anti-inflammatory, anti-angiogenic, and anti-cancer properties, AgNPs have been widely used in various fields. Yan and Chen (2019) reported that AgNPs exposure could inhibit seed germination and root growth, and reduce biomass and leaf area. Also, recent research has shown that spore germination in the ferns *Dryopteris affinis* and *Dryopteris filix-mas* is affected by exposure to AgNPs (Soare et al. 2019).

Lu et al. (2022) observed that concentrations higher than 0.02 mg AgNPs significantly inhibited spore germination of *Ceratopteris thalictroides* fern. Additionally, the proportion of hermaphrodites gametophytes and the area of gametophytes significantly decreased under AgNPs treatment, whereas no significant effect was observed in male gametophytes.

A significant negative correlation between the dry biomass of *Azolla filiculoides* and the AgNPs concentration was reported by López-Herrera et al. (2021). They observed that the relative growth rate at the variant with 0.1 mg AgNPs L<sup>-1</sup> did not have significant differences compared to the control, whereas for the variants with 1, 5, 10 mg AgNPs L<sup>-1</sup> the result is opposite.

The effects of AgNPs on *Physcomitrella patens* vary and are associated with the stability and change of AgNPs in the environment over time. Generally, AgNPs at a dose of 2 µg/mL exhibited an adverse effect (Liang et al., 2018), similar to our observations.

The toxicity of nanoparticles depends on their size (Falco et al., 2020), concentration (Rahman et al., 2023), shape (Gorka et al., 2015), dimensions (Nikzamid et al., 2021), manufacturing process

(Amooaghaie et al., 2015), surface functionalization (Nguyen et al., 2013), route of administration (Wu et al., 2020), experimental model (Alhammad et al., 2023) and organism-specific characteristics (Song and Lee, 2016).

AgNPs' toxicity mechanism is mostly caused by their high ROS release capacity (Hosny et al., 2025), which led to oxidative stress, damage to cell membrane permeability, lipid peroxidation, and changes in cell structure, protein, and DNA damage (Mikesková et al., 2025), and finally to cell apoptosis (Nie et al., 2023). Plants have mechanisms to cope with this stress, but if the antioxidant system (superoxide dismutase, ascorbate peroxidase, catalase, etc.) capacity is exceeded the apoptosis or programmed cell death is inevitable (Muzammil et al., 2023).

AgNPs can also affect cell functions through non-ROS pathways (Das et al., 2021) through the dispersion and delivery of silver ions ( $\text{Ag}^+$ ) (Riaz Ahmed et al., 2017), which can alter the physiological and biochemical functions of cellular components (Noori et al., 2024). Yu et al. (2026) observed that AgNPs nanospheres damaged cell walls, the nanoplates caused cell content leakage, and the nanocubes induced starch granule accumulation. They also suggested that chlorophyll *a* production is stimulated by the low concentration of AgNPs, and the bioaccumulation of AgNPs influences their biotoxicity. AgNPs reduce the mitotic index and disturb the processes of cell division (Kumari et al., 2011), increased micronuclei and chromosomal aberrations (Patlolla et al., 2012), and root growth.

#### 4. CONCLUSIONS

The results obtained indicate that the AgNPs solution, dilution  $10^{-1}$  ( $2\mu\text{g/mL}$ ), inhibits spore germination in the three tested species. The other two nanoparticle solutions (dilutions  $10^{-2}$  and  $10^{-3}$ ) to which the spores and gametophytes were exposed determined a gametophyte differentiation similar to the control variant.

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