

THE TOXICITY OF EXTRACTS WITH BIMETALLIC NANOPARTICLES ON FERNS SPORES GERMINATION

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Abstract

Plants based synthesis of Ag and Au nanoparticles represent one of the most important focus of researchers due to their applications and catalytic activities. We utilized various techniques to obtain extracts with bimetallic nanoparticles from *Asplenium scolopendrium* leaves; we tested the following variants: M - microwave extract, M NP - microwave extract with nanoparticles Au-Ag, C - extract obtained in the oven, C NP - extract obtained in the oven with nanoparticles Au-Ag, H - hydroalcoholic solution; for each extract were made 2 dilutions: 1:10 and 1:100.

The germinative percent was used to quantify the effect of the extracts with/without bimetallic nanoparticles on *Asplenium scolopendrium* and *Dryopteris filix-mas* spores. In the variants with bimetallic nanoparticles, regardless species, dilution or obtaining technique, after 1 week no germination was recorded and after one month the situation was the same. Also, no germination was observed in the variants without bimetallic nanoparticles with 1:10 dilution. In both species, at 1:100 dilution, the variants with the extract obtained using microwave technique (without nanoparticles) recorded a higher germinative percent than the other variants with extract.

Keywords: bimetallic extracts, ferns, spores.

1. INTRODUCTION

Nanotechnology is a rapidly developing industry (Salman Khan et al., 2016) which has a significant influence on society, economy and environment. (Zaka and Abbasi, 2016). Singh et al. (2011) define nanoparticles (NPs) as small objects that behave as a whole unit regarding transport and properties. The classification of nanoparticles can be done upon their morphology, size, chemical properties etc. Based on physical and chemical characteristics Khan et al. (2019) classify nanoparticles in 6 categories: Carbon-based NPs, Metal NPs, Ceramics NPs, Semiconductor NPs, Polymeric NPs, Lipid-based NPs. Metallic nanoparticles have uniform size and their dimensions (length, width, thickness) are within the range 1-100 nm (Harish Kumar et al., 2018); they have unique catalytic, electronic, optical and structural properties (Roopan et al., 2014). For a long time metal nanoparticles have been synthesized using physical and chemical methods (Parsons et al., 2007) but, in the last years, green technology became more attractive (Mohamad et al., 2013). Biological synthesis of nanoparticles is: more energy efficient and able to eliminate the use of dangerous chemicals (Mohamad et al., 2013), relatively simple, cheap (Kulkarni and Muddapur, 2014), ecofriendly and nontoxic. Biosynthesis of nanoparticles using plants systems is under

extensive research (Dauthal and Mukhopadhyay, 2016), plants extracts are preferred over microbial sources because they don't need sterile conditions (Salunke et al., 2014).

Plants based synthesis of Ag and Au nanoparticles represent one of the most important focus of researchers due to their applications and catalytic activities. The Au and Ag metals were used in chronic disease, for enhancing the health and metabolic functions of the human body (Kuppusamy et al., 2017).

Venkatesan and Santhanalakshmi (2012) observed that bimetallic nanoparticles (AuAg) were more efficient than monometallic nanoparticles (Au and Ag) in terms of catalytic activity in the oxidation process of amino acids. Because AuNPs and AgNPs are produced in increasing amounts (Zhan et al., 2019) an important issue is to evaluate their potential toxicity (Barrena et al., 2009). Generally, in biological studies bimetallic nanoparticles are used as antimicrobial and antioxidant agents but in this study we tried a new approach: to determinate the effect of this nanoparticles on the most susceptible stage in ferns development.

2. MATERIALS AND METHODS

Mature leaves from *Asplenium scolopendrium* (As) and *Dryopteris filix-mas* (Dfm) were collected from several individuals in different sites (Vâlsan Valley) in order to ensure spores genetic diversity. After releasing the spores in the sporangia, there followed their collecting and preserving in a refrigerator at 4°C. The extracts were obtained from *Asplenium scolopendrium* leaves which also were collected from Vâlsan Valley. A mixture for extraction (distilled water:ethanol – 1:1) was poured over the chopped leaves and than the solution was kept for 25 minutes at 72°C at microwave (M), and at the same temperature in the oven for 2 and a half hours (C). We utilized various techniques (microwave and the oven) to obtain the extracts because we also wanted to study the effect of obtaining technique extract on the spore germination. Some variants contained, beside the extracts, bimetallic nanoparticles Au - Ag (M NP; C NP). We used AgNO₃ and HAuCl₄ in proportion 1:1. Variants with hydroalcoholic solution were also tested (H). We exposed the spores of the ferns to two different concentration of each extract/solution (1:10, 1:100). For Control the germination media was constituted by distilled water (Table 1).

Table 1. Tested variants

Variants	Contain	Dilution
Control	distilled water	-
M 1:10	microwave extract	1:10
M 1:100		1:100
M NP 1:10	microwave extract with bimetallic nanoparticles (Au-Ag)	1:10
M NP 1:100		1:100
C1:10	extract obtained in the oven	1:10
C 1:100		1:100
C NP 1:10	extract obtained in the oven with bimetallic nanoparticles (Au-Ag)	1:10
C NP 1:100		1:100
H 1:10	hydroalcoholic solution	1:10
H 1:100		1:100

The culture vessels were kept in growth chamber with constant humidity and illumination. The germinative percent was used to quantify the effect of the extracts with/without bimetallic

nanoparticles on *Asplenium scolopendrium* (As) and *Dryopteris filix-mas* spores (Dfm); for obtaining an overview there were made multiple comparisons (with Duncan test) between the variants with extracts and control.

3. RESULTS AND DISCUSSIONS

In the variants with bimetallic nanoparticles, regardless species, dilution or obtaining technique, after 1 week no germination was recorded and after one month the situation was the same (Fig. 3-4). Also, no germination was observed in the variants without bimetallic nanoparticles with 1:10 dilution (Fig.5-6). In both species, at 1:100 dilution, the variants with the extract obtained using microwave technique (without nanoparticles) (Fig. 8) recorded a higher germinative percent than the variants with the extract obtained in the oven (Fig. 1-2, Fig. 7). We can conclude that the microwave technique is more efficient because we had better results using it and the quantity of extract obtained was bigger than that which resulted from the oven. Hydroalcoholic solution inhibited spore germination: in the variants with 1:10 dilution the spores didn't germinate (Fig. 9) while at 1:100 dilution (Fig. 10). the germinative percent was significant smaller than control (Fig. 1-2).

No germination was also reported by Soare et al. (2019) after testing *Asplenium scolopendrium* and *Dryopteris filix-mas* extracts with AgNPs on *Dryopteris filix-mas* and *Dryopteris affinis* spores.

The cytotoxicity of AuNPs varies upon nanoparticles size, shape and surface, aggregation, interaction with biological molecules, cell type and cellular media (Caballero-Díaz and Valcárcel, 2014). AuNPs can induce ROS production which damage DNA and determinate cell death (Jia et al., 2017)

Phytotoxicity of AuNPs (6×10^{-6} M - 1.8×10^{-5} M concentration) was observed in the experiment made by Ostroumov et al (2014) after long term exposure (17 day and more) in *Ceratophyllum demersum*.

Increases in cell death, hydrogen peroxide formation and lipid peroxidation in *Oryza sativa* roots and shoots were observed by Ndeh et al. (2017) after AuNPs exposure, but these increases weren't statistically significant.

It was demonstrated that AgNPs could affect seed germination and root growth, reduce biomass and leaf area, decline transpiration rate and alterate the hormone, disrupt the synthesis of chlorophyll and reduce nutrient uptake (Yan and Chen, 2019).

In *Arabidopsis thaliana* the AgNPs exposure had a significant inhibition on: root elongation in a dose-dependent manner and on chlorophyll content (Qian et al., 2013).

Root and shoot fresh mass and length of *Brassica* sp. seedling decreased in the variants with AgNPs compared with Control. The reduction of growth parameters might be linked with the reduction in photosynthetic performance (Vishwakarma et al., 2017).

The addition of Ag nanoparticles to the nutrient medium decreased seed germination and the growth of shoots and root length in *Vicia faba* (Abd-Alla et al. 2016) while the addition of Au nanoparticles increased the length of stem and root in *Arabidopsis thaliana* (Kumar et al. 2013).

Salman Khan et al. (2016) studied the monometallic (Ag, Au, Cu) and bimetallic (Ag Cu (1:3), Ag Cu (3:1), Au Cu (1:3), Au Cu (3:1), Ag Au (1:3), Ag Au (3:1)) nanoparticles effect on *Silybum marianum*. Seed germination was affected and increased significantly upon treatment.

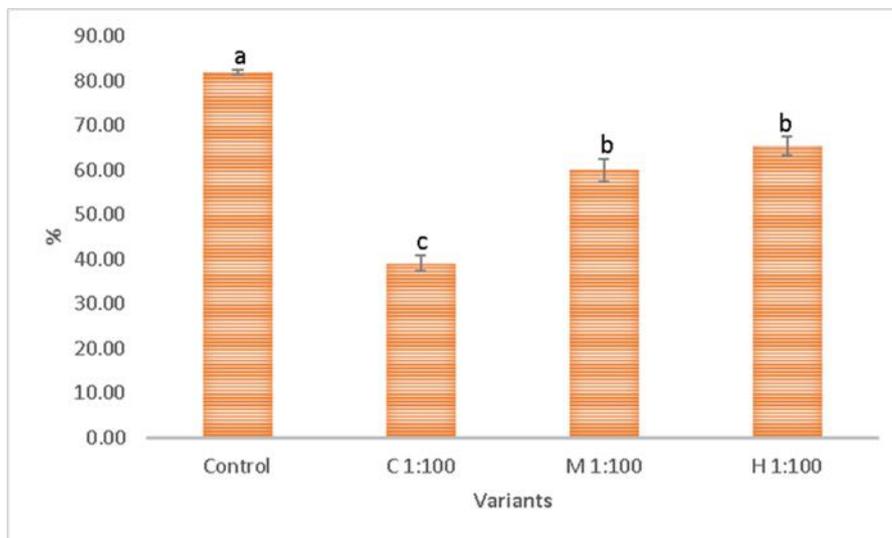


Figure 1. The influence of extracts on spore germination in *Dryopteris filix-mas* – after 1 week

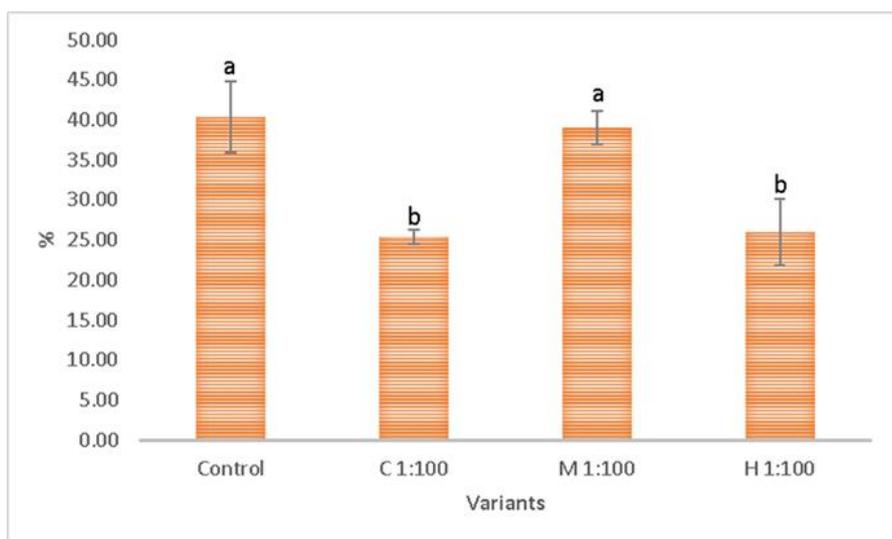


Figure 2. The influence of extracts on spore germination in *Asplenium scolopendrium* – after 1 week

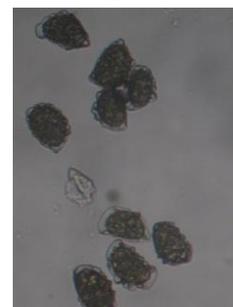
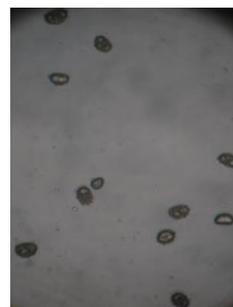
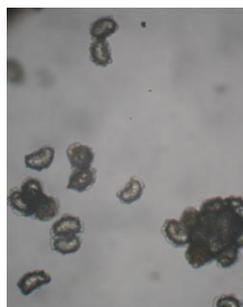


Figure 3. *As C NP 1:100*

Figure 4. *Dfm M NP 1:100*

Figure 5. *As M 1:10*

Figure 6. *Dfm C 1:10*



Figure 7. Dfm C 1:100

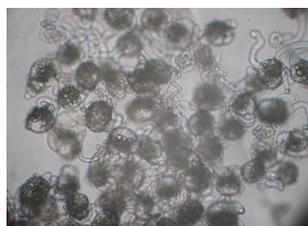


Figure 8. Dfm M 1:100

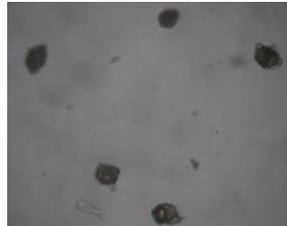


Figure 9. Dfm H 1:10



Figure 10. Dfm H 1:100

Legend: As – *Asplenium scolopendrium*; Dfm – *Dryopteris filix-mas*; The pictures were made after 1 month of exposure (x100)

4. CONCLUSIONS

The *Asplenium scolopendrium* extracts with bimetallic nanoparticles had a negative influence on spores by inhibiting the germination in both ferns species. A similar reaction was observed at the smallest (1:10) dilution in the hydroalcoholic variant and in the variants without nanoparticles.

5. ACKNOWLEDGEMENTS

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