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CHARACTERIZATION IN TERMS OF COMPOSITION AND PHYTOTOXICITY OF AQUEOUS SPORES EXTRACT

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Abstract
In this study, we used aqueous extracts of fern spores and solution of $AgNO_3$ and $HAuCl_4$ for the synthesis of bimetallic nanoparticles Au:Ag in different proportions: 1:1 and 1:10. The spores used come from 2 species of ferns: Asplenium scolopendrium and Dryopteris filix-mas. For the characterization of the extracts with or without bimetallic nanoparticles we applied Fourier transform infrared spectroscopy (FT-IR). Phytotoxicity was tested using Pisum sativum seeds. Each extract was tested in 2 dilutions: 1:10 (D10) and 1:100 (D100). The parameters, which we determinate were the root and stem growth and fresh biomass. Root growth was stimulated in variants with Asplenium scolopendrium extract: without nanoparticles both dilution and with Au:Ag nanoparticles 1:10 D10. The highest values obtained for the stem were at D10 at the variants with Asplenium scolopendrium extract with or without bimetallic nanoparticles. The influence of extracts on fresh biomass was smaller than on the growth of root and stem.

Keywords: bimetallic nanoparticles, extracts, fern spores, FT-IR, Pisum sativum.

1. INTRODUCTION

Because of their unique physical and chemical properties, nanoparticles (NPs) are used in numerous biological, chemical, optical and electronic applications (Rahman et al., 2020). Green synthesis of nanoparticles it is simple, clean, safe and environmentally friendly (Rani et al., 2022). Having antibacterial and biocidal properties, Ag NPs are frequently used in medical application, cosmetics and food services (Jogaiah et al., 2021). AuNPs are easy to synthesize and are stable; they may have different size and relatively low toxicity. AuNPs can be used as an agent for gene/drug delivery because they have small size they can enter in the cell by endocytosis or diffusion (Kumar et al., 2018). Metal combinations are another important subject that has been in the attention of researchers due to their new characteristics different from the properties of the components (Dobrucka et al., 2021). Green synthesized bimetallic Au-Ag nanoparticles have interesting properties and higher catalytic efficiencies than Au and Ag monometallic nanoparticles (Sahu et al., 2020). Ten years before the beginning of the nano-ecotoxicology field, researchers said that NPs can have a negative effect on biota by generating ROS (Khan et al., 2021). Plants are one of the most important forms of life because they are the primary producers in the ecosystem. The effect of nanoparticles on plants varies upon their concentrations, properties (physical and chemical) and

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plant species; various nanoparticles can increase biomass productivity and germination rate (Aqeel et al., 2021). Plants can promote the development of nanotechnology through preparation of nanoparticles (green synthesis) and in turn nanoparticles can promote the health of plants by acting like growth regulators, pesticides, fertilizers, antimicrobial agents, targeted transporters, biosensors (Hu and Xianyu, 2021).
This study determined the capacity of aqueous extracts of fern spores to produce bimetallic Au-AgNPs and to establish the potential toxicity of the extracts with or without nanoparticles.

2. MATERIALS AND METHODS

In this study, we used aqueous extracts of fern spores (*Asplenium scolopendrium - As* and *Dryopteris filix-mas - Dfm)* and solutions of $AgNO_3$ and $HAuCl_4$ for the synthesis of bimetallic nanoparticles Au:Ag in different proportions (1:1 and 1:10). For the characterization of the extracts with or without bimetallic nanoparticles, we applied Fourier transform infrared spectroscopy (FTIR). The spectral measurements were made using a FTIR Jasco 6300 spectrometer with an ATR accessory equipped with a diamond crystal (Pike Technologies). The spectra were recorded in the region of 400-4000 cm^{-1}, detector TGS, apodization Cosine. The spectral data were processed with JASCO Spectra Manager II software.
Phytotoxicity was tested using pea seeds. The seeds of *Pisum sativum* variety Alvesta were bought from Agricultural Research and Development Station Pitești, Albota. The hydration and the immersion in the test solution lasted one hour each. Each extract (Table 1) was tested in 2 dilutions: 1:10 (D10) and 1:100 (D100), and for Control we used distilled water. After immersion in the test solution, the seeds were placed in Petri dishes on filter paper and watered periodically with distilled water. The Petri dishes were placed in the dark until the measurements were made. For each variant, 10 seeds from each plant species were used, and the experiment had three repetitions. After five days from the beginning of the experiment the following parameters were determinate: the growth of root and stem and fresh biomass. With the help of SPSS program - version16 for Windows, we calculated the average and compared them with Duncan test.

Table 1. Tested variants

Variants	Contain
Control	Distilled water
AA M	Aqueous extract obtained from As spores
AA 1:1	Aqueous extract obtained from As spores with Au-Ag NPs 1:1
AA 1:10	Aqueous extract obtained from As spores with Au-Ag NPs 1:10
DA M	Aqueous extract obtained from Dfm spores
DA 1:1	Aqueous extract obtained from Dfm spores with Au-Ag NPs 1:1
DA 1:10	Aqueous extract obtained from Dfm spores with Au-Ag NPs 1:10

3. RESULTS AND DISCUSSIONS

In extracts with nanoparticles, the carbonyl group at 1635 cm^{-1} shows an increased intensity as a result of the capture/reduction of the metals. It was also confirmed that the carbonyl group from the protein and amino acid had stronger ability to bind with metal nanoparticles or act as stabilizing agents (Table 2, Fig. 1-6).
A difference was witnessed for two peaks at 1419 cm^{-1} and 3225 cm^{-1} in the case of AA 1:1 and AA1:10.

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The signals at frequencies above 3345-3255 cm^{-1} can be assigned to those molecules that are involved in hydrogen-bonding interactions. The data in Figures 4-6 and Table 2 show distinct differences for the different nanoparticles. As the solvent water, exhibits systematically varying dielectric constants because of their joint hydroxyl group and the remaining group with decreasing polarity (Kiefer and col., 2015). Hence, they can interact with the nanoparticle surface via different mechanisms such as hydrogen-bonding, polar interactions, and van der Waals forces. The OH band is highly sensitive to changes in the hydrogen-bonding network, so a difference was witnessed the peak at 3225 cm^{-1} in the case of AA 1:1 (3241 cm^{-1}) and AA1:10 (3255 cm^{-1}).
Root growth in *P. sativum* was stimulated in variants with *Asplenium scolopendrium* extract: without nanoparticles both dilution and with Au:Ag NPs 1:10 D10 (Fig.7a). The highest values obtained for the stem were at D10 at the variants with *Asplenium scolopendrium* extract with or without bimetallic nanoparticles (Fig.7b). The results recorded, both for root and stem, for the rest of variants were similar to the control or smaller, but the differences were not significant. The influence of extracts on fresh biomass was smaller than on root and stem growth (Fig.7c).
In the experiment performed by Rani et al. (2021) the plants from Control were similar to the plants from the variants with AgNP, and the concentration of nanoparticles within plants was low. At low concentrations, AgNPs can have a positive effect on *Pisum sativum* on protein and carbohydrate content (Mehmood and Murtaza, 2017), fresh and dry biomass and photosynthesis (Prażak et al., 2020) and root length (Barabanov et al., 2018).

Table 2. FTIR

	FTIR band absorption peaks (cm^{-1})					Assignments
DA M	**DA 1:1**	**DA 1:10**	**AA M**	**AA 1:1**	**AA 1:10**	
722	723	722	722	723	722	Out-of-plane bending vibrations (Schulz and Baranska, 2007)
1338	1339	1339	1338	1339	1339	CH_2 wagging (Yang et al., 2005; Lucassen et al., 1998; Mordechai et al., 2000)
1397	1397	1397	1419	1397	1397	δCH3
1435	1435	1435	1435	1435	1435	δ (CH_2) (polysaccharides, cellulose)
1456	1456	1456	1456	1456	1456	Asymmetric CH_3 bending modes of the methyl groups of proteins (Shetty et al., 2006)
1473	1473	1473	1473	1473	1473	CH_2 bending of the methylene chains in lipids
1488	1488	1488	1488	1488	1488	C=C, deformation C-H (Shetty et al., 2006)
1507	1507	1507	1507	1507	1507	In-plane CH bending vibration from the phenyl rings (Schulz and Baranska, 2007)
1540	1540	1540	1540	1540	1540	Protein amide II absorption- predominately β-sheet of amide II
1558	1558	1558	1558	1558	1558	CO stretching (Wang et al., 1997) Predominately a-sheet of amide II (Amide II band mainly stems from the C-N stretching and C-N-H bending vibrations weakly coupled to the C=O stretching mode) (Eckel et al., 2001)
1636	1635	1636	1636	1636	1635	Amide I band (arises from C=O stretching vibrations) (Huleihel et al., 2002)
1730	1732	1732	1732	1732	1732	C=O band
3245	3269	3269	3255	3241	3255	Stretching O-H

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Labeeb et al. (2020) observed that all seedling growth parameters in *Pisum sativum*, except the root length, were reduced after AgNPs exposure. AgNPs with size over 22 nm, in 1000-3000µM concentration, produced a decline in plant growth and synthesis of plant pigments in *Pisum sativum* (Tripathi et al., 2017).

The cytotoxicity of AuNPs varies upon their concentration, particle size and shape. At lower concentration they stimulate vegetative growth and improved free radical scavenging potential and antioxidant enzymatic activities (Siddiqi and Husen, 2016). According to Zhu et al. (2012) AuNPs uptake and distribution depend on both nanoparticle surface charge and plant species. Positively charged AuNPs are taken up by plant roots, while negatively charged AuNPs are translocated into plant stems.

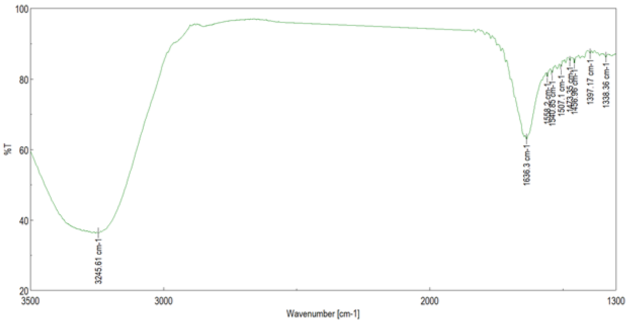

Fig.1 ATR-FTIR spectrum DA M

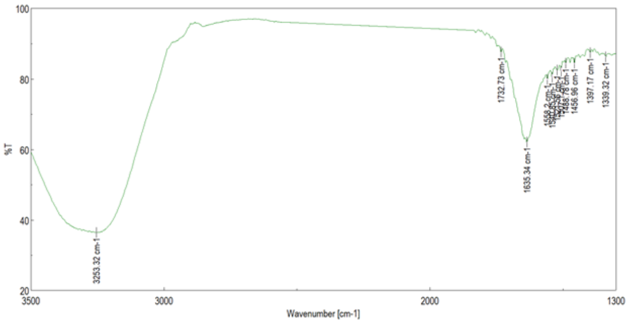

Fig.2 ATR-FTIR spectrum DA 1:1

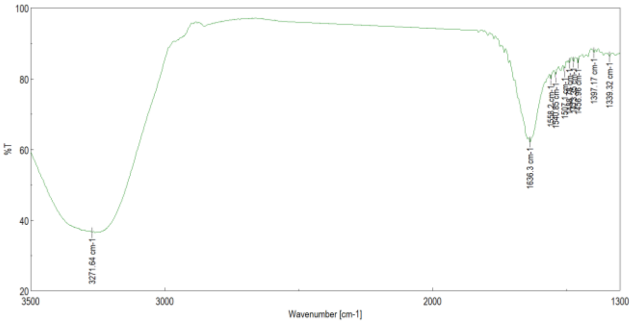

Fig.3 ATR-FTIR spectrum DA 1:10

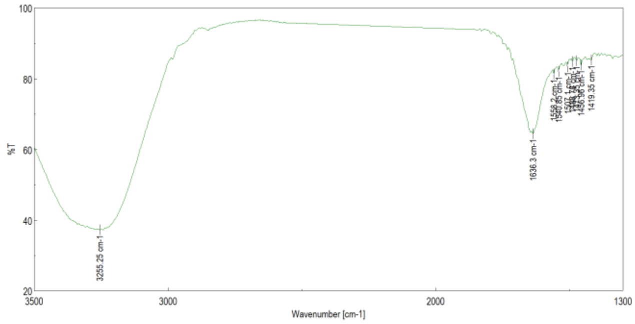

Fig.4 ATR-FTIR spectrum AA M

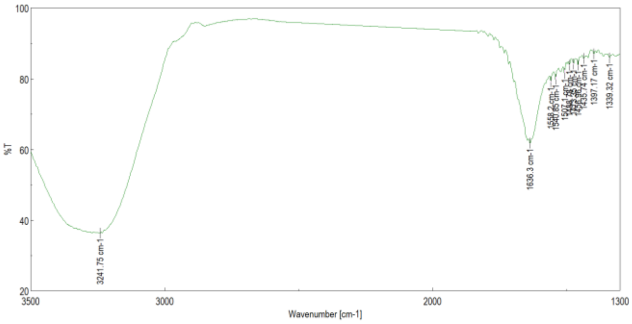

Fig.5 ATR-FTIR spectrum AA 1:1

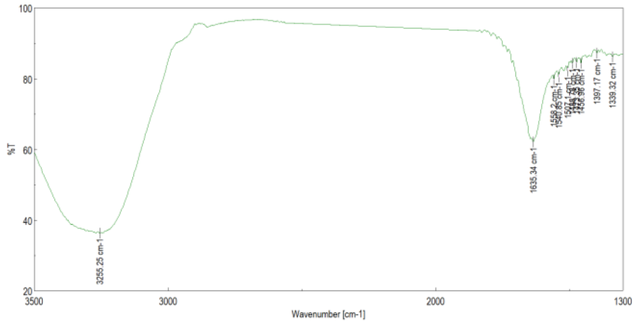

Fig.6 ATR-FTIR spectrum AA 1:10

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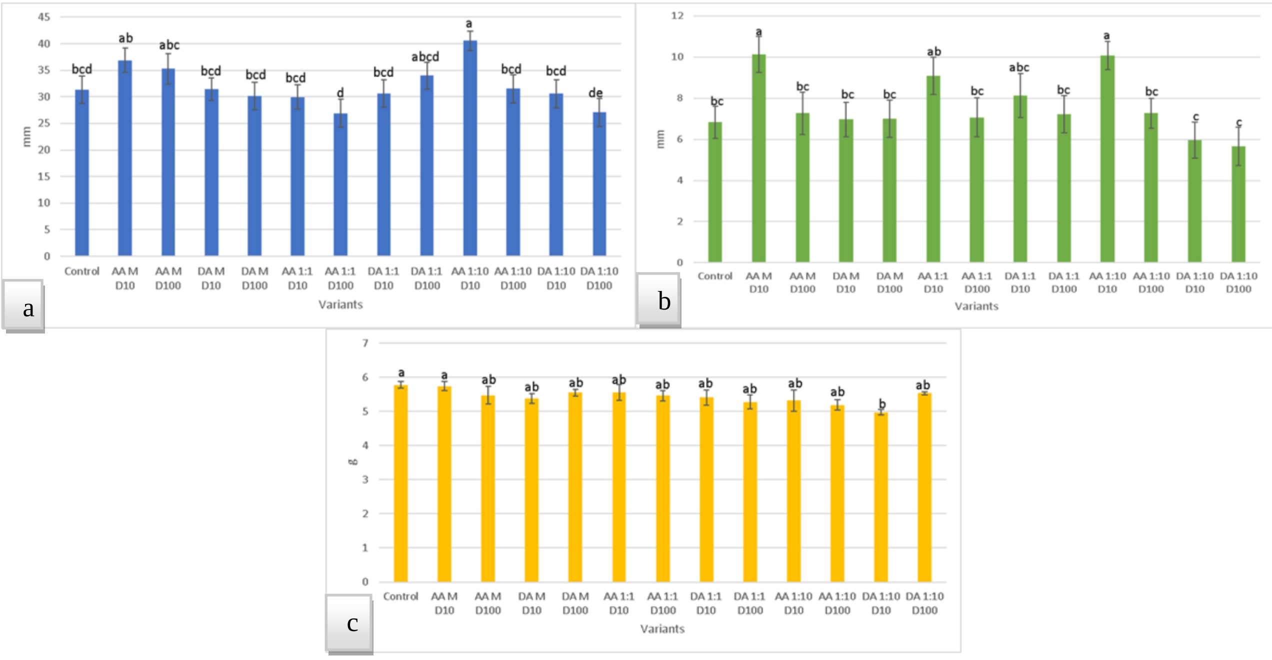

Fig.7 Extracts influence on Pisum sativum: a) root growth; b) stem growth; c) fresh biomas

Ndeh et al. (2017) observed a slight decrease in rice root and shoot lengths compared to the control, after AuNP exposure. The root lengths of *Arabidopsis thaliana* seedlings were reduced, after AuNPs exposure, by 75% (Taylor et al., 2014), while at *Brassica juncea* the influence on growth parameters was positive (Arora et al., 2012).

AuNPs and AgNPs didn`t stimulate the germination of seeds in *Ficus* plants, but they had a positive impact on *Mimusops laurifolia* seedlings by enhancing leaf growth (Alshehddi and Bokhari, 2020).

Jurkow et al. (2020) studied the antioxidant potential of oakleaf lettuce seedlings sprayed with AuNPs and AgNPs in different concentrations. They observed that the total peroxidase activity increased after applying Au-NPs, but decreased after Ag-NPs treatment, while the carotenoid content increased in both treatments.

At high concentrations, Ag, Au and Cu nanoparticles showed a high level of toxicity and increased production of secondary metabolites, phenolic content, flavonoids, antioxidant activity, total protein content (Hussain et al., 2017).

The effect of bimetallic nanoparticles varies upon species: Ag/AuNPs were most toxic to *Lepidium sativum* and least toxic to *Linum flavum*, while for *Zea mays*, *Solanum lycopersicum* var. *cerasiforme* and *Salvia hispanica* seeds the phytotoxicity was similar (Szymaski and Dobrucka, 2020). After the nanoparticles penetrate the seeds, the effects they cause on the germination and growth of the seeds are long-lasting; the changes can be significant and can affect the biochemical profile (Hussain et al., 2018).

Ghosh et al. (2021) reported no phytotoxic effect of the biosynthesized AuNPs and Au–Ag NPs (≤100 µg/ml) on lentil seeds, while AgNPs exhibited a little phytotoxicity at higher (100 µg/ml) concentration level.

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4. CONCLUSIONS

The reduction of silver nitrate and chloroauric acid to silver nanoparticles and gold, respectively, was confirmed by the FTIR spectroscopy technique. The lowest phytotoxicity was observed at the variants with *A. scolopendrium* extracts 1:10 dilution, where was observed a root and stem growth stimulation.

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