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THE EFFECT OF SPORES EXTRACTS WITH GREEN SYNTHESIZED BIMETALLIC NANOPARTICLES ON *CUCUMIS SATIVUS* L.

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Key words: *extracts, green synthesis, bimetallic nanoparticles, cucumber*

INTRODUCTION

The global population grows rapidly and along with it the requirement to satisfy the nutrional demands (Mittal et al., 2020) in a way that can ensure food security and safety (Bakht et al., 2021). In this situation, the scientist try developing new techniques to boost plants native functions (Sadak, 2019): enhance nutrient uptake, improve plant growth and sustained release of agrochemicals (Narware et al., 2019). The development of nanotechnologies represents a promising way to improve sustainable agriculture because nanoparticles can be used as fertilizers ($KFeO_2$NPs, FeO_x NPs and MnO_xNPs (Saleem et al., 2021; De França Bettencourt et al., 2020), fungicides (CuONPs and ZnONPs) (Sardar et al., 2022), herbicides, insecticides (SiNP) (Caceres et al., 2019), can reduce stress caused by various factors (salinity stress is reduced in *Triticum* with SiO_2NP (Mushtaq et al., 2019) and AgNPs (Wahid et al., 2020) application) or can be used for seed priming. Green synthesis is an important method to produce nanoparticles due to the lack of toxic waste and because is relatively simple and cheap; it has been used more and more every day due to its ecological nature (Mahesh et al., 2022), cost-effective, reproducibility, and energy efficiency compared to physical or chemical syntehis (Hammad et al., 2022). However, nanoparticles have unique properties that influence their bioavailability, bioaccumulation and toxicity. NP effect on plants depends on plant species, growth stage, type of nanoparticle, concentration (Goswami et al., 2019), exposure time, size, surface structure (Chen et al., 2018), shape, coating, use of carrier (Cox et al., 2017). Because of this situation, ecotoxicological research, which studies the effect of nanoparticles are important. Besides the effect of nanoparticles on plants, ecotoxicology also studies the exposure and accumulation at different levels (Tlili and Mouneyrac, 2021). Cox et al. (2017) consider that root elongation studies are more effective in phytotoxicity measuring compared to seed germination studies. The AgNPs toxicity can be observed from seedling stage to fully grown plants (Yin et al., 2012). Bimetallic nanoparticles (Au-Ag) are more reactive than monometallic nanoparticles, so the evaluation of their toxicity is important for sustainable use (Ghosh et al., 2022).

In this study, we determined the effect of spore extracts with green synthesized bimetallic nanoparticles on *Cucumis sativus* L.

MATERIAL AND METHOD

Extracts. For obtaining the extracts, we used spores from two species of ferns, *Asplenium scolopendrium* L. (As) and *Dryopteris filix-mas* (L.) Schott (Dfm), and two types of solvents (water and hydroalcoholic solution). The bimetallic nanoparticles (Au:Ag 1:1 and Au:Ag 1:10) were synthesized using $HAuCl_4$ and $AgNO_3$.

Experimental variants. After hydration, the *Cucumis sativus* L. seeds were immersed in the test solution for one hour. The extracts were tested after being diluted 10 and 100 times, respectively (Table 1-2). For Control we used distilled water and for the variant Hidro – hidroalcoholic solution (1:1 water and ethanol). After the treatment the seeds were placed in Petri dishes on filter paper and watered periodically with distilled water.

Table 1. Variants for hydroalcoholic extracts

VARIANTS	CONTENT	Dilution
Control	Distilled water	-
Hidr 10	Hydroalcoholic solution	10
Hidr 100		100
DH M D10	Hydroalcoholic extract of Dfm spores	10
DH M D100		100
DH 1:1 D10	Hydroalcoholic extract of Dfm spores with bimetallic nanoparticles Au-Ag 1:1	10
DH 1:1 D100		100
DH 1:10 D10	Hydroalcoholic extract of Dfm spores with bimetallic nanoparticles Au-Ag 1:10	10
DH 1:10 D100		100
AH M D10	Hydroalcoholic extract of As spores	10
AH M D100		100
AH 1:1 D10	Hydroalcoholic extract of As spores with bimetallic nanoparticles Au-Ag 1:1	10
AH 1:1 D100		100
AH 1:10 D10	Hydroalcoholic extract of As spores with bimetallic nanoparticles Au-Ag 1:10	10
AH 1:10 D100		100

The Petri dishes were placed in the dark until the measurements were made. In the 6th day at the beginning of the experiment, we measured the root and stem length with graph paper. After we determinate the fresh biomass the plant material was put in the oven, at 80 °C, for establish the dry biomass. For each variant we used 10 seeds. Data obtained after 3 repetitions were statistical analyzed using IBM SPSS Statistics 23 programs. The mean and standard error were calculated, and the averages were compared with Duncan test. The results from Duncan test are mentioned in the charts with letters (a, b, c, d etc).

Table 2. Variants for aqueous extracts

VARIANTS	CONTENT	Dilution
Control	Distilled water	-
DA M D10	Aqueous extract of Dfm spores	10
DA M D100		100
DA 1:1 D10	Aqueous extract of Dfm spores with bimetallic nanoparticles Au-Ag 1:1	10
DA 1:1 D100		100
DA 1:10 D10	Aqueous extract of Dfm spores with bimetallic nanoparticles Au-Ag 1:10	10
DA 1:10 D100		100
AA M D10	Aqueous extract of As spores	10
AA M D100		100
AA 1:1 D10	Aqueous extract of As spores with bimetallic nanoparticles Au-Ag 1:1	10
AA 1:1 D100		100
AA 1:10 D10	Aqueous extract of As spores with bimetallic nanoparticles Au-Ag 1:10	10
AA 1:10 D100		100

RESULTS AND DISCUSSIONS

The influence of hydroalcoholic extracts on Cucumis sativus seeds

The hydroalcoholic extracts with Au:AgNPs 1:1dilution 10 inhibited both the root (Fig.1) and stem growth; the inhibition of the stem was significant for both variants AH 1:1 D10 and DH 1:1 D10 compared to control (Fig.6). While Au:AgNPs present in the hydroalcoholic extracts from As and Dfm spores diluted 100 times had a positive effect on the growth in length of the root (Fig.2-5). For these variants were obtained higher values than the one determined at control.

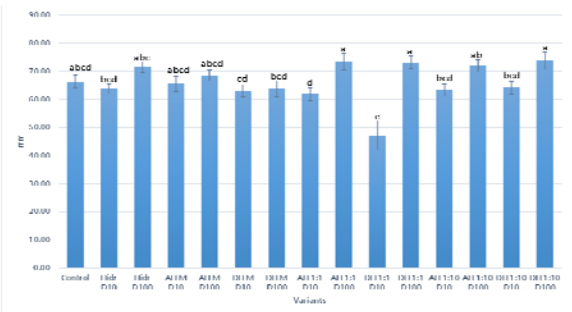

Fig. 1. The influence of hydroalcoholic extracts on root growth

In the case of the stem, this stimulation is maintained only in the variants with Dfm extract with Au:AgNPs 1:1 and 1:10 dilution 100 (Fig.6). Growth of root and stem at variants DH 1:1 D100, DH 1:10 led to the growth of fresh biomass, without any significant differences between these variants and the control (Fig.7). The smallest value for dry biomass was obtained in control (Fig.8).

Fig.2 AH 1:1 D100 - *C. sativus*

Fig.3 DH 1:1 D100 - *C. sativus*

Fig.4. AH 1:10 D100 - *C. sativus*

Fig.5 DH 1:10 D100 - *C. sativus*

Fig. 6. The influence of hydroalcoholic extracts on stem growth

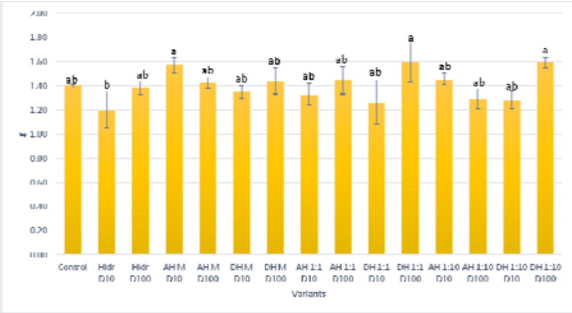

Fig. 7. The influence of hydroalcoholic extracts on fresh biomass

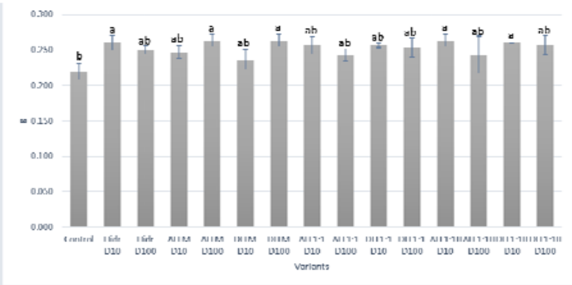

Fig. 8. The influence of hydroalcoholic extracts on dry biomass

The influence of aqueous extracts on Cucumis sativus seeds

Exposure of *Cucumis sativus* seeds to aqueous extracts of As and Dfm spores with and without bimetallic nanoparticles has generally stimulated root growth (Fig.9), while the stem growth was stimulated only at the variants without bimetallic nanoparticles (Fig.10)

The values recorded for fresh biomass did not determine the presence of significant differences between variants (Fig.11).

Aqueous extracts of *Asplenium scolopendrium* and *Dryopteris filix-mas* leaves with and without Ag nanoparticles stimulated the growth *of C. sativus* roots compared to the control (Soare et al., 2020).

After AgNPs exposure, an increase in the germination rate was observed in case of *Cucumis sativus* seeds (Sotoodehnia et al., 2019).

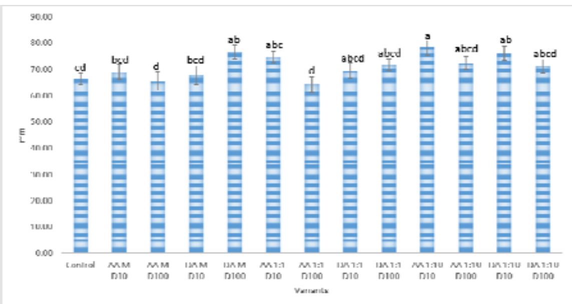

Fig. 9. The influence of aqueous extracts on root growth

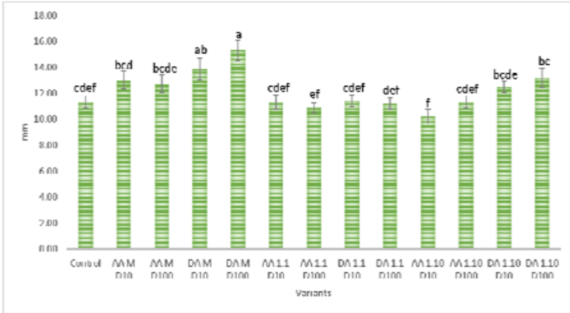

Fig. 10. The influence of aqueous extracts on stem growth

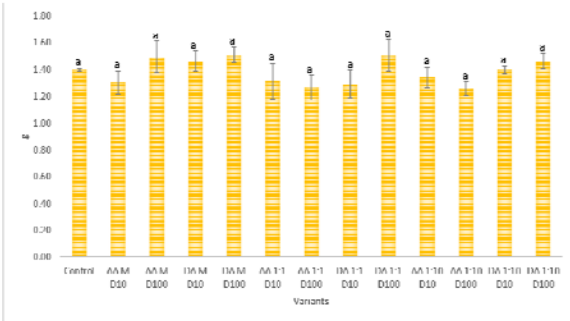

Fig. 11. The influence of aqueous extracts on fresh biomass

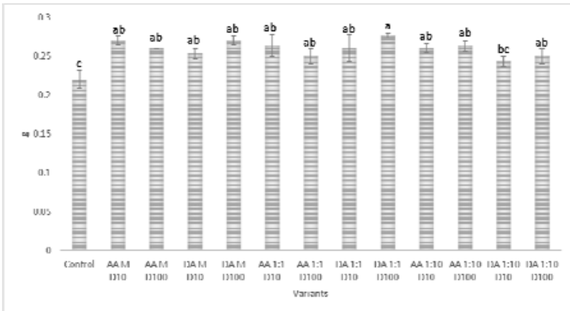

Fig. 12. The influence of aqueous extracts on dry biomass

Cui et al. (2014) observed that cucumber root elongation was stimulated after exposure to Ag nanoparticles in concentrations lower than 20 mg L^{-1}, and after 3 weeks of exposure, the dry root biomass was considerably reduced in the 50 and 100 mg L^{-1} variants.

In the experiment performed by Shams et al. (2013) the increase in the concentration of Ag nanoparticles increased fruit production in *Cucumis sativus*, and in the variant with a concentration of 3000 ppm Ag nanoparticles, an increase in dry biomass was observed compared to the control. AgNPs can stimulate germination, root elongation, biomass growth and increase yield of seeds by influencing the uptake of nutrients, increasing the water uptake into seeds (Landa, 2021).

AgNPs, concentration 100 µg mL^{-1}, had a negative influence on cucumber seeds roots growth, while FeNPs, concentration 116 µg mL^{-1}, and AuNPs, concentration 62 µg mL^{-1}, showed no significant differences compared to control (Barrena et al., 2009).

The influence of AgNPs on cucumber seedlings growth was studied by Tripathi et al. (2017) in terms of root and stem length, fresh and dry biomass. They observed that increasing the AgNPs concentration reduced the growth parameters, which can be a result of AgNPs accumulation and ROS production.

Ghosh et al. (2022) observed no phytotoxic effect on germination of lentil seed after the treatments with biosynthesized AuNPs or bimetallic Au-AgNPs at all concentrations, while AgNPs exhibited phytotoxic effect at 100 µg mL^{-1}.

CONCLUSIONS

The toxic potential of the extracts varied upon to: type of extract (hydroalcoholic/aqueous), the spores species (As/Dfm), the dilution and the determined parameter. The highest phytotoxicity was observed in case of hydroalcoholic extract at DH 1:1 D10 (root and stem), the differences between this variant and control were significant.

For the aqueous extract, the inhibition was smallest than for the hydroalcoholic extract; even if for the variants AA 1:1 D100 (root) and AA 1:10 D10 (stem) were recorded values below control the differences are minor. Fresh biomass was the parameter that had the fewest variations.

ABSTRACT

The development of nanotechnologies represents a promising way to improve sustainable agriculture because nanoparticles can be used as fertilizers, fungicides, herbicides, insecticides, can reduce the stress caused by various factors (salinity) or can be used for seed priming. Green synthesis is an important method to produce nanoparticles due to the lack of toxic waste and because is relatively simple and cheap. However, nanoparticles have unique properties that influence their bioavailability, bioaccumulation and toxicity.

Because of this ecotoxicological research, which studies the effect of nanoparticles on plants are important. In this study, we determined the effect of spore extracts with green synthesized bimetallic nanoparticles on cucumber. For obtaining the extracts, we used spores from two species of ferns (*Asplenium scolopendrium* and *Dryopteris filix-mas*) and two types of solvents (water and hydroalcoholic solution).

The bimetallic nanoparticles (Au:Ag 1:1 and Au:Ag 1:10) were synthesized using $HAuCl_4$ and $AgNO_3$. The extracts were tested in *Cucumis sativus* seeds after being diluted 10 and 100 times, respectively. Exposure of *Cucumis sativus* seeds to aqueous extracts of *A. scolopendrium* and *D. filix-mas* spores with and without bimetallic nanoparticles has usually stimulated root growth, while the stem growth was stimulated only at the variants without bimetallic nanoparticles. For the hydroalcoholic extracts, at the variants with extracts with bimetallic nanoparticles diluted 100 times were obtained better results than in control with water or with hydroalcoholic solutions.

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