Higher Concentration of Silver Nano Particles Inhibits Spore Germination, Growth and Development of the Gametophyte of *Ceratopteris richardii.*

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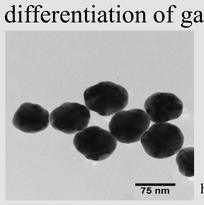
Introduction:

Silver nanoparticles (AgNP) are used in society's daily life through drug delivery[1], personal care products like clothes, cosmetic products[2], and much more which can have a negative effect in the environment. For this project the model organism *Ceratopteris richardii* was used which is commonly known as C-fern. The reason this model organism was used because of its short and distinct life cycle. Just like all other plants *C. richardii* also show alternation of generations (sporophyte and gametophyte phase) [3] but both the phases are properly visible. In one study they found that the rate of spore germination decreased when it was exposed to higher concentrations of AgNPs[4]. In another study they found that the percentage of hermaphrodite gametophyte compared to male gametophyte also decreased as the concentration of nanoplastics, another environmental pollutant, increased[5]. The purpose of our study was to look at the effects of AgNPs on spore germination and gender differentiation of gametophytes in *Ceratopteris richardii* .



https://www.fishersci.com/shop/products/ag-np-75nm-pvp-dried-1mg/501959454

Research Questions and Hypothesis:

- **Research Questions:**

1. Does AgNPs inhibit spore germination?
2. Does the percentage of hermaphrodites decrease when exposed to AgNPs?

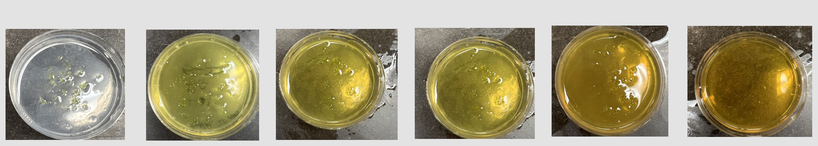
- **Hypothesis:**

H_1: AgNPs will inhibit spore germination of *C. richardii.*

H_2: AgNPs will have a negative effect on the percentage of hermaphrodites of *C. richardii.*

Materials and Methods:

- Spores were sown on Parker agar medium with Thompson micronutrients and supplemented with AgNPs (0ppm, 20ppm, 40ppm, 60ppm, 80ppm, 100ppm) and maintained in an incubator at 28°C under continuous light.
- Cultured spores were examined under an Olympus SZH10 dissecting microscope to observe and calculate the germinated spores starting 3 days from sowing (dfs) until 17 dfs. Spores were considered germinated if primary rhizoid was observed.
- After 22dfs the numbers of hermaphrodites and male gametophytes were counted, recorded, and imaged under an Olympus SZH10 dissecting microscope.
- Wet mounts were made and only the female hermaphrodites across all the concentrations were imaged under a Labomed Lx 300 compound microscope equipped with an Accu-Scope SKYE 2 Wifi camera and software.
- Statistical analysis of the data was determined by Student's t-Test with significant level as 0.05.



0ppm 20ppm 40ppm 60ppm 80ppm 100ppm

Results:

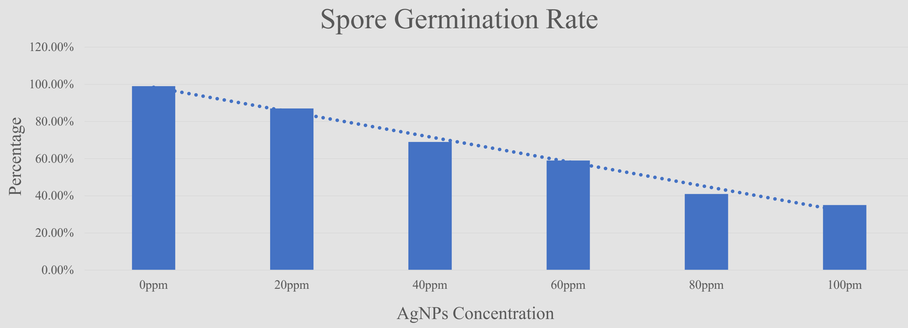


Figure 1 : Graph of spore germination- As the concentrations of AgNPs increased, the percentage of spore germination decreased which is represented by the dotted line.

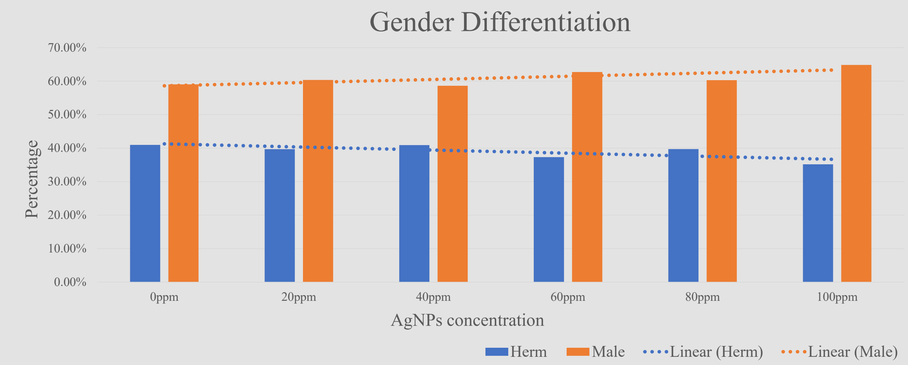


Figure 2 : Graph of gender differentiation- The percentage of male gametophytes across the concentrations of AgNPs were not different, from the control (orange dotted line), whereas the percentage of hermaphrodites were significantly different, $P<0.05$ from the control (blue dotted line).

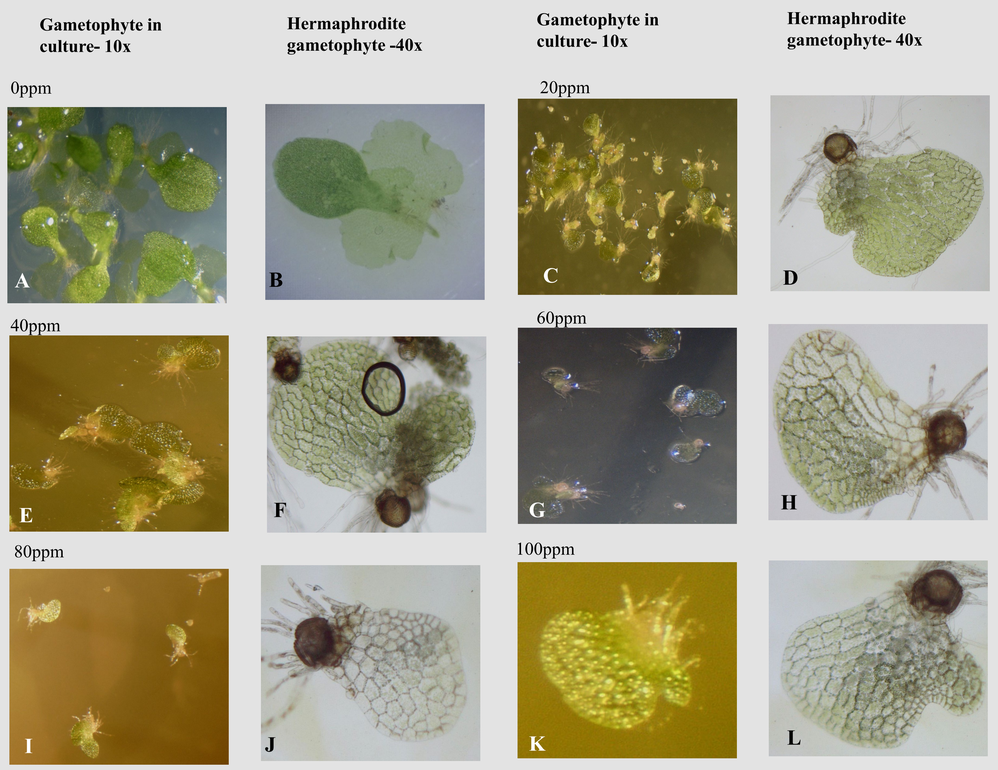


Figure 3: Light microscope images of 20 day old gametophytes in culture (A, C, E, G, I, K) and 22 day old hermaphrodite from each concentration of AgNPs (B, D, F, H, J, L). (A) Hermaphrodites from control had been fertilized showing the first leaf of the sporophyte generation. (B) Hermaphrodite of control with first leaf and root of developing sporophyte. All hermaphrodites in AgNP treatments (C-L) were delayed in there growth compared to the control, and therefore, were not matured enough to undergo fertilization by day 22.

Discussion and Conclusions:

- We found that all concentrations of AgNPs (20-100ppm) inhibits spore germination in *C. richardii* (Figure 1).
- Additionally, the percentage of male gametophytes across the concentrations of AgNPs were not significantly different from the control, whereas the percentage of hermaphrodites in *C. richardii* were (Figure 2).
- Compared to the controls the growth of the hermaphrodites in the treatments were delayed by at least a week (Figure 3).
- Ecological consequences of having fewer hermaphrodites gametophytes would translate to fewer sporophytes, the diploid phase responsible for producing spores.
- These findings are comparable to other studies that have looked at the effect of AgNPs on spore germination and gender differentiation [4].
- For future studies we would like to look at how AgNPs effect the whole life cycle including fertilization rates, embryo development, sporophyte and root growth.

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- We also appreciate Mr. William Browning for technical assistance.

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