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Research Article

Effects of biogenic silver nanoparticles applied to *Nepeta cataria* L. seeds on sterilization and germination

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Abstract: Plant tissue culture applications are carried out under sterile conditions. Culture media must be created without biological contamination and must be maintained aseptically. Contaminations that occur in the culture media may affect experimental results by preventing adequate nutrition and the development of plants. Contamination is mostly caused by microorganisms found on the surfaces of plant tissues used in culture processes. For this reason, explants must be subjected to sterilization processes before culture processes. However, sterilants used for this purpose may have toxic effects on plant tissues, and in recent years, there has been a need to discover effective sterilants that do not show toxic effects. This study aimed to determine the potential of silver nanoparticles (AgNP) in surface sterilization processes for sterile *in vitro* germination of *Nepeta cataria* L. seeds. In order to determine the most appropriate concentration and time to be applied to plant seeds, five different concentrations (0, 75, 100, 125, 150 mg/L) and three different times (5, 10, 20 min) were tested. As a result of the applications, it was determined that the lowest contamination was in the seeds that were kept in 70% ethyl alcohol for 3 min and then in AgNP solutions at a concentration of 150 mg/L for 20 min. No toxicity symptoms were observed in the plants obtained. The results show that AgNPs can be used to obtain *in vitro* sterile plants.

1. INTRODUCTION

Sterile conditions are necessary for success in plant tissue culture applications. Creating these conditions is possible by subjecting the starting material to an effective sterilization process. Otherwise, microorganisms coming from the surface or inside of the plant materials in the nutrient medium multiply and consume the nutrient elements in the tissue culture medium, preventing the growth and development of the plants. Chemicals such as sodium hypochlorite, ethyl alcohol, mercury chloride, hydrogen peroxide, and silver nitrate are used to sterilize plant tissues taken from plants grown in field or greenhouse conditions for use in tissue culture. After using these sterilizers, the explants are washed with sterile distilled water and transferred to the nutrient medium. However, these chemicals used for sterilization can damage living tissues. Therefore, there is a need to discover new generation sterilizing agents (Kocaçalışkan, 2021).

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Nanoparticles have emerged as an alternative way to find solutions to the problems of infectious diseases caused by different pathogenic bacteria and the increase in antibiotic-resistant bacteria in recent years. It is important to investigate the potential of nanoparticles, which have found widespread use because of rapid and comprehensive developments in technology, in plant tissue culture applications (Kim *et al*., 2007). Nanoparticles are clusters of atoms with dimensions of 1-100 nm. Studies in which nanoscale materials are discovered as new antimicrobial agents due to their high surface area/volume ratio and unique physical and chemical properties attract attention in the literature (Rai *et al*., 2009). Although there is little information about their effects and risks on the environment and human health, they have found use in many areas such as chemistry, medicine, biotechnology, biomedicine, cosmetology, electrochemical sensors, and biosensors (Beykaya & Çağlar, 2016; Oyar, 2014; Tunca, 2015).

In order for nanoparticles to have an antibacterial effect, they must be in contact with bacterial cells. Their contact with bacteria occurs through electrostatic attraction, Van der Waals forces, receptor-ligand and hydrophobic interactions. After contacting bacteria, nanoparticles pass through the bacterial membrane and disrupt the shape and function of the cell membrane. Later, nanoparticles cause oxidative stress, heterogeneous changes, electrolyte imbalances caused by impaired cell membrane permeability, and interaction with basic cellular components of bacteria such as DNA, lysosomes, ribosomes and enzymes, causing enzyme inhibition and protein deactivation (Kırmusaoğlu & Cansız, 2018).

It is reported that metal particles such as copper, zinc, titanium, and gold do not have antimicrobial properties, while silver has the most excellent effect against microscopic organisms, infections, and other eukaryotic microorganisms (Duncan, 2011). The fact that silver is a very broad-spectrum antibiotic and has almost no bacterial resistance has led to many studies to discover its nanoscale capabilities (Cheon *et al*., 2019; Manosalva *et al*., 2019; Siddiqi *et al*., 2018).

Nepeta cataria L. (catnip) is an aromatic perennial plant belonging to the genus *Nepeta* of the family Lamiaceae and is well known for its medicinal and therapeutic values. It has served as the representative plant of this genus as it is the most studied species of this genus (Fazil & Porwal, 2022). In addition to its characteristic effects on cats, which give catnip its common name, natural products obtained from this plant are widely known as arthropod repellents (Gomes *et al*., 2024). Traditionally, the plant has been used as a remedy for fever, cold, cough, stomach problems, diarrhea, sore throat, headache, pneumonia, women's problems, blood disorders, convulsions, rheumatism, nervousness, insomnia, hypertension, and toothache (Sharma *et al*., 2019). Researchers have found them to be antifungal, antibacterial, antioxidant, insecticidal, anti-inflammatory, antinociceptive, and potentially spasmolytic. Volatile oils, flavonoids, phenolic acid, steroids, terpenoids, and terpenoid hydrocarbons have all been identified in this plant (Nadeem *et al*., 2022).

In this study, the potential use of silver nanoparticles as sterilizers to prevent contamination problems in plant tissue culture applications was investigated. The seeds of the medically and economically important plant *Nepeta cataria* were treated with silver nanoparticles (AgNP) at different concentrations and times, and the effects of these particles on sterilization and seed germination performance were investigated.

2. METHOD

The medically important plant *Nepeta cataria* was used in the study. The seeds of the plant were provided by Kütahya Hekim Sinan Medicinal Plants Research Center. AgNPs were synthesized by the green method using green tea (*Camellia sinensis* L.) leaf extract. Green tea extract was obtained by the classical brewing method with 2 g of tea leaves in deionized water at 70 °C. 200 mg/L $AgNO_3$ solution and green tea extract were incubated for 24 h, and the nanoparticles were spun in a centrifuge at 5000 rpm for 10 min. The precipitated AgNPs were then dispersed in 25 mL of deionized water. A certain amount of the prepared nanoparticles

was dissolved by heating 2.0 mL of HNO_3. The concentration of the particles was determined as 158 mg/L by Atomic Absorption Spectrometry (AAS, Perkin Elmer Optima 9100).

Sterilization studies were conducted by first soaking *N. cataria* seeds in 70% ethyl alcohol for 3 minutes, followed by immersion in silver nanoparticle solutions at concentrations of 0, 75, 100, 125, and 150 mg/L for durations of 5, 15, and 20 minutes. In order to remove the sterilants in the seeds, they were soaked in 3 series of sterile distilled water for 3 min each and then rinsed. The seeds, which were placed on sterile blotting paper to remove excess water, were transferred to jars containing Murashige and Skoog (MS) (Murashige and Skoog, 1962) nutrient medium.

MS nutrient medium containing 0.1 mg/L inositol, 3% sugar, and 0.7%g agar was used for germination of seeds. The pH of the nutrient medium was adjusted to 5.7 using 0.1 M HCl and 0.1 M NaOH, and after adding 0.7% g agar, they were sterilized by autoclaving at 121 ^oC, 1.06 kg/cm^2 pressure for 15 min. All culture procedures were carried out in the plant growth room under 25± 2^o C and 4000 lux white fluorescence light, 16 hours of light and 8 hours of dark conditions.

Applications were made in 10 culture vessels for each concentration and application time. Germination and contamination results in a total of 150 culture vessels were statistically evaluated. Data were collected using a randomized plot design with five replications. Data obtained from the applications were evaluated using the SPSS 16.0 statistical program (SPSS Inc., Chicago, USA).

3. RESULTS

In the study, the effect of AgNPs applied at different concentrations and durations on *in vitro* sterilization and germination of *N. cataria* seeds was investigated. For this purpose, applications were made at five different concentrations: 0, 75, 100, 125, 150 mg/L and three different durations: 5, 10, 20 min. As a result of the seed germination processes, it was observed that the first germination was on the twelfth day, and the germination rate was determined to be 31%. The data obtained were compared statistically to determine the differences between the applications.

Nanoparticle applications applied to seeds show a statistically significant difference according to the concentration and time variables of contamination observed in seeds (Table 1) (F4-145 Concentration = 6.496, $p< .05$; F4-145 Time = 6.133, $p< .05$). Tukey HSD, which is a multiple comparison test, was performed to determine at which time degrees there was a difference. Contamination values in nanoparticle sterilization processes applied to seeds at different times were determined as 0 mg/L 70% ($\bar{X} = .53$), 100 mg/L 33.3% ($\bar{X} = .40$), and 75 mg/L 16.6% ($\bar{X} = .36$).

Table 1. ANOVA results regarding the effects of applied nanoparticle concentration amounts and durations on the contamination observed in *N. cataria* seeds.

Variable	Subgroup (mg/L-min)	*N*	Mean	*SS.*	*Sd.*	*F*	*p*
Concentration (mg/L)	0	30	0.70	0.49	4-145	6.496	.00*
	75	30	0.16	0.37			
	100	30	0.33	0.47			
	125	30	0.30	0.46			
	150	30	0.43	0.50			
Time (min.)	5	50	0.24	0.43	2-147	6.133	.003*
	10	50	0.38	0.49			
	20	50	0.54	0.50			

The times of nanoparticle application to seeds show a significant difference in germination performances (Table 2) (F4-145 Time = 5.828, $p< 0.05$). Tukey HSD multiple comparison tests

were performed to determine at which time point there was a difference. The effects of AgNP sterilization treatments applied to seeds at different times on germination were determined as 45% ($\overline{X}$ = 4.16) for 20 minutes, 37.5% ($\overline{X}$ = -.22) for 5 minutes, and 27.5% ($\overline{X}$ = -.26) for 10 minutes.

Table 2. ANOVA results regarding the effects of applied nanoparticle concentration amounts and durations on the germination performance of *N. cataria* seeds.

Variable	Subgroup (mg/L-min)	*N*	Mean	*SS.*	*Sd.*	*F*	*p*
Concentration (mg/L)	0	30	0.26	0.44	4-145	2.319	.06
	75	30	0.20	0.40			
	100	30	0.10	0.30			
	125	30	0.36	0.49			
	150	30	0.36	0.49			
Time (min.)	5	50	0.32	0.47	2-147	5.828	.004*
	10	50	0.36	0.48			
	20	50	0.10	0.30			

4. DISCUSSION and CONCLUSION

In order to achieve success in plant tissue culture, the sterilization process must be carried out effectively. Otherwise, the presence of viruses, bacteria, and fungi on the surfaces of plant materials used in *in vitro* studies may cause contamination in the nutrient medium and the death of plant materials in the culture medium (Ramalashmi *et al*., 2018). The sterilization processes to be applied to purify the surface of explants to be used in culture studies from bacteria, fungi, and similar organisms are different. Depending on the method used in sterilization, chemicals such as sodium hypochlorite, ethyl alcohol, silver nitrate, hydrogen peroxide, etc., can be used (Bharti *et al*., 2018; Murthy *et al*., 2019). Today, with the increase in nanotechnological applications and usage areas, there are many studies in which nanomaterials are used for surface sterilization. Recent studies have shown that surface sterilization of explants using nanoparticles significantly reduces microbial contamination (Mandeh *et al*., 2012; Taghizadeh and Solgi, 2014; Bao *et al*., 2022).

In this study, the potential use of AgNPs in surface sterilization for *in vitro* germination of *N. cataria* seeds was investigated. For this purpose, five different concentrations of AgNPs, 0 mg/L, 75 mg/L, 100 mg/L, 125 mg/L, 150 mg/L, and three different durations, 5 min, 10 min, and 20 min were applied to plant seeds. Observations were made because of the applications, and the obtained data were compared statistically. As a result of the observations, the first germination of the seeds was seen on the twelfth day, and the germination rate was determined as 31%. When the literature is examined, it was reported that the germination performance was between 30% and 80% in a study conducted with *N. cataria* seeds (Aćimović *et al*., 2021). In another study conducted by Ibrahim *et al*. (2017), the germination rate of *N. cataria* seeds without stratification application was reported as 21%. The germination rate obtained as a result of our study shows that the seed successfully germinated at a higher rate under plant tissue culture conditions than the applications reported in the literature without the stratification application. The study also determined that nano-sized silver can prevent contamination in tissue culture conditions and that the use of AgNP solutions in surface sterilization has an acceptable effect in controlling contamination without any negative effects on *N. cataria* seed germination and developmental characters. However, no difference was determined between the concentrations applied to control bacterial contamination. As a result of the observations, it was determined that nanoparticle application caused morphological changes such as growth in leaves, elongation in plant height, and stem thickening, and positively affected the development of the plant. These observation results are consistent with many studies reporting that nanoparticles play a regulatory role in plant growth with positive effects such as increasing

chlorophyll content, stomatal conductance, and net photosynthesis rate (Rhaman *et al*., 2022; Siddiqi and Husen, 2022; Verma *et al*., 2024). These results indicate that AgNPs should be investigated for their effects on plant development in addition to their use in surface sterilization processes.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Burcu Çetin: Supervision and Validation, Research, Resources, Writing-original draft. **Aslı Aktay**: Methodology, Writing, Research. **Çiğdem Ay**: Methodology, Writing, Research.

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