

Effects of Silver Nanoparticles on Germination and Seedling Characteristics of Oriental Beech (*Fagus orientalis*) Seeds

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Nowadays, the applications of nanotechnology are increasing in various fields such as information technology, energy, the medical sector, and agriculture. Nanotechnology has proved its ability to solve problems in agriculture and related industries. Establishing the impact of nanoparticles on various ecosystems has become a primary research topic, but studies on forest ecosystems and trees are quite limited. This study examined the effects of silver nanoparticles on the germination parameters of oriental beech seeds and established their toxic threshold values. Silver nanoparticles were applied at concentrations of 200, 400, 600, 800, and 1000 mg/L to oriental beech (*Fagus orientalis*) seeds collected from 10 different populations in order to identify the germination rate, germination percentage, seedling height, root collar diameter, plumula length, radicle thickness, and radicle length parameters. The results revealed that silver nanoparticles have a negative effect on germination and seedling parameters of oriental beech seeds, and that this effect is clearly seen in the germination rate at 20 mg/L levels and in seedling characters starting from 60 mg/L dose, causing a decrease of 13% in germination rate, 24% in germination percentage, 40% in plumula length, and 30% in radicle length. The Kahramanmaraş-Andirin population was found to be the most affected by nanoparticles, while the Bursa-Inegöl and Ordu-Akkus populations were the least affected.

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INTRODUCTION

Rapid industrial developments have created the need for raw material supply required for production, and the extraction and use of underground mines caused by this have led to an increase in the concentrations of many elements in receiving environments such as soil, water, and air (Arıcak *et al.* 2019; Key *et al.* 2023). These developments in the industrial field have caused significant changes in population projections and balances in the ecosystem. Urbanization due to the concentration of the population in urban areas (Dogan *et al.* 2022; Zeren Cetin *et al.* 2023) and global climate change due to changes in the composition of the atmosphere (Tekin *et al.* 2022; Varol *et al.* 2022a,b) have become irreversible problems (Koc *et al.* 2023).

In addition to these problems, environmental pollution is the most significant problem caused by technological and industrial developments (Kuzmina *et al.* 2023).

Pollution, caused by heavy metals used as raw materials in industry, is seen as the most important problem threatening human and environmental health worldwide (Sulhan *et al.* 2022; Yayla *et al.* 2022). This is because heavy metals are elements, many of which can be toxic, carcinogenic, and fatal to humans even at low concentrations, and even those, which are necessary as nutritional elements, are unhealthy at high concentrations (Ucun Ozel *et al.* 2020; Karacocuk *et al.* 2022). The elements remain intact in nature for a long time, making them even more hazardous (Cesur *et al.* 2022). The concentrations of heavy metals are constantly increasing due to anthropogenic factors (Cobanoglu *et al.* 2023a).

Recent technological developments have taken the threat of heavy metal pollution to a new level. Silver nanoparticles, as well as magnetic field sensors, infrared (IR) detectors, photoconductors, light-emitting diodes, sun-selective coatings, various sensors, solar cells, and photo detectors are commonly used (Afsheen *et al.* 2020). Nanoparticle pollution is a more advanced type of pollution that contains all concerns of heavy metal pollution. Particularly in the chip and information technologies, nanoparticles are used in the development of products obtained from natural elements and minerals during the production of devices, which have domestic and industrial use. Nanoparticle pollution is explained as a level of pollution formed by the particles that emerge during production and damage all biological life cycles by slowly bringing them to the non-operating and unanalysable level of functionality (Cañas *et al.* 2008).

Nanotechnology is being applied in various fields including information technology, energy, the medical sector, and agriculture (Sadak *et al.* 2008). Nanoparticle pollution affects all living systems. It damages biological functions in animal, plant, and human societies. However, despite these hazardous effects, the use of nanoparticles and the products containing them is rapidly increasing due to the advancement of nanotechnologies in the sector of new material use. Along with the effects of global warming in recent years, nanoparticles, which enter the atmosphere and rapidly spread in the environment, continue to cause serious harm to life by reaching even the most remote and inaccessible parts of the globe. The number of studies conducted on nanoparticle pollution formations as well as their harms can be described as insufficient (Ozdemir 2023). Some of the most important effects of nanoparticles are observed in forests. Identifying the effects of nanoparticles on forest ecosystems and forest elements is of great importance in terms of revealing the extent of the danger as well as taking the necessary precautions. This study aimed to identify the effects of silver (Ag) nanoparticles, which are increasingly spreading in nature and causing nanoparticle pollution, at different doses on the germination parameters of the seeds of oriental beech (*Fagus orientalis*). The hypothesis of the study can be explained as “Ag nanoparticles affect the germination ability and seedling characters of beech seeds”. Oriental beech is one of the important primary forest tree species in Türkiye, and it was collected from different locations. Oriental beech is an important wood raw material and its seeds are very important for wildlife due to their high nutrient content (Ayaz *et al.* 2011; Hrivnak *et al.* 2023).

EXPERIMENTAL

Materials and Method

The seeds that were used were collected from natural beech forests of Türkiye. Information regarding the populations from which the seeds were collected is given in Table 1 and Fig. 1.



Fig. 1. Locations of populations

The seeds were subjected to health tests, and the oriental beech seeds, which showed no problems in the parts of embryo and endosperm and had normal and healthy developmental performance, were used in germination tests.

Table 1. Populations from which the Seeds Were Collected

Pop. No	Region	Coordinates		Altitude (m)	Exposure	Stand Type
P1	Adapazari-Karasu	41° 01' 13"	30° 44' 45"	300-500	Northwest	Knd ₂
		41° 00' 38"	30° 45' 01"			
P2	Balikesir-Dursunbey	39° 27' 35"	28° 33' 54"	800-1000	North	Knd ₁
		39° 27' 30"	28° 32' 44"			
P3	Bartın-Kumluca	41° 29' 43"	32° 26' 32"	450-700	North	Knd ₃
		41° 29' 55"	32° 27' 54"			
P4	Bursa-Inegöl	39° 58' 44"	29° 29' 04"	700-1200	Northwest	Knd ₃
		39° 58' 02"	29° 28' 46"			
P5	Canakkale-Kalkım	39° 46' 56"	27° 09' 46"	400-600	North	Knd ₂
		39° 46' 11"	27° 09' 10"			
P6	Düzce-Yığılca	40° 58' 54"	31° 27' 26"	700-1100	Northwest	Knd ₃
		40° 59' 47"	31° 25' 35"			
P7	Kahramanmaraş-Andirın	37° 46' 12"	36° 22' 10"	1400-1800	North	Knd ₃
		37° 44' 52"	36° 23' 34"			
P8	Karabük-Yenice	41° 09' 08"	32° 16' 37"	600-900	North	Knd ₁
		41° 08' 52"	32° 15' 53"			
P9	Ordu-Akkus	40° 47' 25"	36° 58' 50"	1100-1400	North	Knd ₂
		40° 46' 56"	36° 59' 05"			
P10	Zonguldak-Devrek	41° 12' 27"	31° 53' 15"	500-700	Northwest	Knd ₁
		41° 16' 13"	32° 12' 52"			

One of the primary aims of the study was to reveal the effects of Ag nanoparticles on the germination parameters of oriental beech seeds and to determine their toxic threshold values. For this purpose, 5 different nanoparticle concentrations at the doses of

20, 40, 60, 80, and 100 were prepared under sterile and hygienic conditions in the laboratory environment and kept in sterile concentration bottles to be applied to the collected seeds.

For germination tests, the oriental beech seeds, to which five different doses of concentrations were applied, were placed in disposable sterile petri dishes prepared with quantitative filter papers. Five repetitions were performed for each dose, and including 30 healthy seeds for each repetition, nanoparticles were applied to 150 seeds in total. A total of 900 seeds, including the control group, were used during germination tests. Germination tests were carried out in a 3M ClimaCell brand germination cabinet. In the germination cabinet, the temperature of the germination environment was set as 20 °C, relative humidity as 70%, and exposure time as 12 h.

Germination was monitored by applying 10 mL of nanoparticle solution daily to the oriental beech seeds placed in 100 mL petri dishes in a way not to touch each other. On the 7th day of the applications, the number of seeds germinated was counted in order to calculate the germination rate (GR). The application continued for 35 days, and at the end of the 35th day, seedling length (SeedL), root collar diameter (RCD), plumula length (PIL), radicle length (RadL), and radicle thickness (RadT) were measured using a digital micro-compass in all germinated seeds. The seeds, which had not been germinated, were cut and checked whether they were healthy or not, and the germination percentage (GP) was calculated by dividing the total germinated seeds to the total healthy seeds. Similarly, the germination rate was found by dividing the number of seeds, germinated on the 7th day, to the number of healthy seeds. The obtained data were evaluated with the help of the SPSS 22.0 package program, and analysis of variance and Duncan test were applied to the data.

RESULTS AND DISCUSSION

When examining the results, it was found that the application doses on GR were not statistically significant in all populations, except P7. At the same time, the population-based changes in GR was not statistically significant at all doses, except the control group and 600 mg/L. The lowest GR value was obtained at 1000 mg/L dose of nanoparticle application, while the highest value was obtained at 200 mg/L dose of nanoparticle application, in all populations. However, the GR value was observed to be decreasing as the nanoparticle application increases.

Table 2. Changes in GR Depending on Population and Nanoparticle Application

	Control	200	400	600	800	1000	F	MEAN
P1	19.1 ^{abcd}	19.6	19.0	18.7 ^{abc}	17.7	17.2	0.6 ^{ns}	18.6 ^b
P2	20.2 ^{bcde}	21.1	20.2	19.6 ^{bc}	18.3	18.1	0.4 ^{ns}	19.6 ^{bc}
P3	18.1 ^{ab}	17.5	16.1	15.8 ^{ab}	15.6	14.6	1.2 ^{ns}	16.3 ^a
P4	21.1 ^{de}	22.1	22.4	20.8 ^c	20.7	19.1	0.3 ^{ns}	21.0 ^{cd}
P5	18.7 ^{abc}	19.9	18.8	18.9 ^{abc}	17.7	16.8	0.6 ^{ns}	18.5 ^b
P6	19.8 ^{abcde}	20.2	20.0	19.6 ^{bc}	19.2	17.7	0.3 ^{ns}	19.4 ^{bc}
P7	17.8 ^a	15.5 ^{AB}	15.7 ^{AB}	15.1 ^{aA}	13.7 ^A	13.9 ^A	3.6 [*]	15.3 ^a
P8	20.7 ^{cde}	22.5	21.1	20.6 ^c	20.0	18.7	0.7 ^{ns}	20.6 ^{cd}
P9	21.9 ^e	22.2	23.4	21.8 ^c	20.4	19.5	0.6 ^{ns}	21.5 ^d
P10	18.5 ^{ab}	17.3	17.0	16.4 ^{ab}	16.0	15.3	0.8 ^{ns}	16.7 ^a
F	4.1 ^{**}	1.9 ^{ns}	1.8 ^{ns}	2.9 [*]	1.9 ^{ns}	1.8 ^{ns}		13.2 ^{***}
MEAN	19.6 ^C	19.8 ^C	19.4 ^{BC}	18.7 ^{BC}	17.9 ^{AB}	17.1 ^A	4.3 ^{**}	

On the basis of population, the lowest value was obtained in P7, while the highest value was obtained in P9. In the control group, while the lowest value was obtained in P7 (17.8%), the highest was obtained in P9 (21.9%). In P7, it decreased to 15.5% at 200 mg/L dose of nanoparticle application and this number was observed to be gradually decreasing. In P9, GR increased to 22.2% at 200 mg/L dose of nanoparticle application, but decreased to 19.5% at 1000 mg/L dose of nanoparticle application depending on the dose.

Table 3. Changes in GP Depending on Population and Nanoparticle Application

	Control	200	400	600	800	1000	F	MEAN
P1	68.4 ^{bc}	61.9 ^{bcdBC}	61.8 ^{abBC}	56.4 ^{bcdAB}	49.5 ^{abcA}	49.9 ^{bcdA}	8.8 ^{***}	58.0 ^{cde}
P2	71.5 ^{bcd}	63.7 ^{cdC}	63.8 ^{bC}	60.6 ^{cdeBC}	55.0 ^{bcdAB}	53.2 ^{cdeA}	9.7 ^{***}	61.3 ^{efg}
P3	59.2 ^a	56.7 ^{abBC}	56.4 ^{abC}	51.5 ^{abB}	44.5 ^{aA}	43.0 ^{abA}	14.5 ^{***}	51.9 ^{ab}
P4	75.2 ^{cd}	65.5 ^{cdA}	65.6 ^{bA}	62.6 ^{deA}	59.9 ^{deA}	60.7 ^{eA}	7.0 ^{***}	64.9 ^{gh}
P5	65.9 ^{ab}	60.6 ^{abcdBC}	61.4 ^{abBC}	55.7 ^{bcAB}	48.7 ^{abA}	48.9 ^{abcdA}	7.0 ^{***}	56.9 ^{cd}
P6	69.9 ^{bc}	62.5 ^{bcdB}	61.2 ^{abB}	58.0 ^{bcdAB}	54.2 ^{bcdA}	53.7 ^{cdeA}	9.5 ^{***}	59.9 ^{def}
P7	57.6 ^a	54.7 ^{aBC}	54.8 ^{aBC}	46.7 ^{aAB}	42.8 ^{aA}	41.0 ^{aA}	5.1 ^{**}	49.6 ^a
P8	74.5 ^{bcd}	63.4 ^{cdAB}	64.7 ^{bB}	60.1 ^{cdeAB}	56.4 ^{cdeA}	56.5 ^{deA}	8.5 ^{***}	62.6 ^{fgh}
P9	78.7 ^d	67.2 ^{dB}	66.1 ^{bB}	64.7 ^{eAB}	61.5 ^{eA}	60.6 ^{eA}	20.4 ^{***}	66.5 ^h
P10	59.7 ^a	59.4 ^{abcB}	59.4 ^{abB}	55.5 ^{bcAB}	48.4 ^{abA}	47.6 ^{abcA}	3.4 [*]	55.0 ^{bc}
F	7.0 ^{***}	3.4 ^{**}	2.8 [*]	6.1 ^{***}	7.6 ^{***}	6.9 ^{***}		14.9 ^{***}
MEAN	68.1 ^D	61.6 ^C	61.5 ^C	57.2 ^B	52.1 ^A	51.5 ^A	38.2 ^{***}	

According to the results of variance analysis, the application doses were statistically significant in all populations on GP. Likewise, the population-based changes in GP were found to be statistically significant at all doses. In general, the lowest GP value in all populations was obtained at high dose (1000 mg/L) of nanoparticle application, while the highest GP value was obtained in the control group. The GP value decreased as the nanoparticle concentration increased.

On the basis of population, the lowest value was generally obtained in P7, while the highest value was obtained in P9. In the control group, the lowest value was obtained in P7 (57.6%), while the highest value was obtained in P9 (78.7%). In P7, it decreased to 54.7% at 200 mg/L dose of nanoparticle application, and it continued to decrease as the dose increased. In P9, GP decreased to 67.2% at 200 mg/L dose of nanoparticle application, and continued to decrease in the same way.

Table 4. Changes in Seedling Length, Depending on Population and Nanoparticle Application

	Control	200	400	600	800	1000	F	MEAN
P1	7.0 ^{bc}	7.4	7.4 ^b	7.0	6.9	6.6	1.5 ^{ns}	7.1 ^{bcd}
P2	7.1 ^{bc}	7.5	7.5 ^b	7.3	7.1	6.8	0.8 ^{ns}	7.2 ^{def}
P3	6.4 ^{ab}	7.1	7.1 ^{ab}	6.7	6.6	6.3	1.0 ^{ns}	6.7 ^{ab}
P4	7.2 ^c	7.7	7.7 ^b	7.5	7.4	7.0	0.8 ^{ns}	7.4 ^{ef}
P5	6.9 ^{abc}	7.3	7.3 ^b	6.8	6.7	6.5	0.8 ^{ns}	6.9 ^{bcd}
P6	7.1 ^{bc}	7.4	7.5 ^b	7.3	7.1	6.7	2.3 ^{ns}	7.2 ^{cdef}
P7	6.3 ^a	6.8	6.5 ^a	6.6	6.4	6.3	0.2 ^{ns}	6.5 ^a
P8	7.2 ^c	7.6	7.5 ^b	7.4	7.3	6.9	0.7 ^{ns}	7.3 ^{ef}
P9	7.2 ^c	7.8	7.8 ^b	7.6	7.4	7.2	0.9 ^{ns}	7.5 ^f
P10	6.6 ^{abc}	7.2	7.2 ^{ab}	6.7	6.7	6.5	1.0 ^{ns}	6.8 ^{abc}
F	2.3 [*]	0.8 ^{ns}	2.2 [*]	1.3 ^{ns}	1.0 ^{ns}	0.7 ^{ns}		7.1 ^{***}
MEAN	6.9 ^{AB}	7.4 ^D	7.3 ^{CD}	7.1 ^{BC}	7.0 ^B	6.7 ^A	7.6 ^{***}	

Taking the results obtained into consideration, it was seen that the application doses were not statistically significant with respect to SeedL in all populations. Likewise, the population-based change in SeedL was not found to be statistically significant at all doses, except the control group. The lowest SeedL value was obtained at 1000 mg/L dose of nanoparticle application in all populations, while the highest value was obtained at 200 mg/L dose of nanoparticle application. The SeedL value was observed to be decreasing as the nanoparticle concentration increases.

Table 5. Changes in RCD Depending on Population and Nanoparticle Application

	Control	200	400	600	800	1000	F	MEAN
P1	0.8	0.8	0.8	0.8	0.7	0.7	0.2 ^{ns}	0.8 ^{abcd}
P2	0.8	0.9	0.9	0.9	0.8	0.8	0.4 ^{ns}	0.8 ^{cd}
P3	0.7	0.8	0.8	0.7	0.8	0.7	0.2 ^{ns}	0.8 ^{abc}
P4	0.9	0.9	0.8	0.9	0.8	0.8	0.5 ^{ns}	0.8 ^{cd}
P5	0.8	0.8	0.9	0.8	0.7	0.7	0.6 ^{ns}	0.8 ^{abcd}
P6	0.8	0.9	0.7	0.8	0.8	0.7	1.2 ^{ns}	0.8 ^{abcd}
P7	0.7	0.8	0.7	0.7	0.7	0.8	0.1 ^{ns}	0.7 ^{ab}
P8	0.8	0.8	0.9	0.8	0.8	0.8	0.3 ^{ns}	0.8 ^{bcd}
P9	0.9	0.9	0.8	0.8	0.9	0.8	0.3 ^{ns}	0.8 ^d
P10	0.8	0.8	0.7	0.7	0.7	0.7	0.2 ^{ns}	0.7 ^a
F	0.6 ^{ns}	0.4 ^{ns}	0.7 ^{ns}	0.6 ^{ns}	1.0 ^{ns}	0.3 ^{ns}		2.7 ^{**}
MEAN	0.8	0.8	0.8	0.8	0.8	0.7	1.4 ^{ns}	

While the lowest population-based value was obtained in P72, the highest was obtained in P9. In the control group, the lowest value was obtained in P7 (6.3%), while the highest value was obtained in P4, P8, and P9 (7.2%). In P7, SeedL, which increased to 6.8% at 200 mg/L dose of nanoparticle application, began to decrease inversely with the dose increase, and decreased to 6.3% at 1000 mg/L dose of nanoparticle application. In P9, SeedL increased to 7.8% at 200 mg/L dose of application and continued to decrease immediately afterwards depending on the dose increase. When examining the results given in the table, it is apparent that the application doses were not statistically significant in all populations on RCD. At the same time, the population-based changes in RCD were not statistically significant at all doses. Overall, on a population basis, the lowest value was obtained in P10, while the highest value was obtained in P9.

Table 6. Changes in Pumula Length, Depending on Population and Nanoparticle Application

	Control	200	400	600	800	1000	F	MEAN (Nano1)
P1	1.0 ^{bcd}	0.7 ^A	0.7 ^A	0.7 ^A	0.6 ^A	0.6 ^A	12.5 ^{***}	0.7 ^{abcd}
P2	1.1 ^{cd}	0.7 ^A	0.7 ^A	0.7 ^A	0.7 ^A	0.6 ^A	12.8 ^{***}	0.8 ^{bcd}
P3	0.9 ^{ab}	0.7 ^A	0.7 ^A	0.6 ^A	0.6 ^A	0.6 ^A	4.1 ^{**}	0.7 ^{ab}
P4	1.1 ^{cd}	0.8 ^A	0.8 ^A	0.7 ^A	0.7 ^A	0.6 ^A	12.2 ^{***}	0.8 ^{cd}
P5	1.0 ^{abcd}	0.7 ^A	0.7 ^A	0.6 ^A	0.6 ^A	0.6 ^A	5.9 ^{**}	0.7 ^{abcd}
P6	1.0 ^{cd}	0.7 ^A	0.7 ^A	0.7 ^A	0.6 ^A	0.6 ^A	11.6 ^{***}	0.7 ^{abcd}
P7	0.8 ^a	0.6 ^A	0.7 ^A	0.6 ^A	0.6 ^A	0.6 ^A	5.3 ^{**}	0.7 ^a
P8	1.1 ^{cd}	0.8 ^B	0.8 ^B	0.7 ^{AB}	0.7 ^{AB}	0.6 ^A	15.6 ^{***}	0.8 ^{bcd}
P9	1.1 ^d	0.8 ^A	0.8 ^A	0.7 ^A	0.7 ^A	0.7 ^A	12.9 ^{***}	0.8 ^d
P10	0.9 ^{abc}	0.7 ^A	0.7 ^A	0.6 ^A	0.6 ^A	0.6 ^A	7.9 ^{***}	0.7 ^{abc}
F	3.3 ^{**}	0.9 ^{ns}	1.1 ^{ns}	0.7 ^{ns}	1.0 ^{ns}	0.3 ^{ns}		2.5 ^{**}
MEAN	1.0 ^D	0.7 ^C	0.7 ^C	0.7 ^{BC}	0.6 ^{AB}	0.6 ^A	87.4 ^{***}	

According to the results of variance analysis, the application doses with respect to PIL were statistically significant in all populations. The population-based changes in PIL were not statistically significant at all doses, except the control dose. Generally, in all populations, the lowest PIL value was obtained at the 1000 mg/L dose of nanoparticle application, while the highest value was obtained in the control group. The PIL value was observed to be decreasing as the nanoparticle concentration increased. On a population basis, the lowest value was generally obtained in P7, while the highest value was obtained in P9. In the control group, while the lowest value was obtained in P7 (0.8%), the highest value was obtained in P9 (1.1%).

Table 7. Changes in Radical Thickness, Depending on Population and Nanoparticle Application

	Control	200	400	600	800	1000	F	MEAN
P1	1.1 ^{bcd}	1.1	1.0	1.1	0.9	0.9	1.7 ^{ns}	1.0 ^{abc}
P2	1.2 ^{cde}	1.2 ^B	1.0 ^{AB}	1.0 ^{AB}	1.0 ^{AB}	0.9 ^A	3.0 [*]	1.1 ^{abc}
P3	1.0 ^{ab}	1.1	1.0	0.9	1.0	0.9	1.1 ^{ns}	1.0 ^{ab}
P4	1.2 ^{de}	1.1	1.1	1.1	1.1	1.1	1.1 ^{ns}	1.1 ^c
P5	1.1 ^{abc}	1.2	1.1	1.0	1.0	1.0	1.8 ^{ns}	1.1 ^{abc}
P6	1.2 ^{cde}	1.1 ^B	1.0 ^{AB}	1.0 ^{AB}	1.0 ^{AB}	0.9 ^A	2.6 [*]	1.0 ^{abc}
P7	1.0 ^a	1.0	1.0	1.0	1.0	0.9	0.2 ^{ns}	1.0 ^a
P8	1.2 ^{cde}	1.1	1.1	1.1	1.0	0.9	1.7 ^{ns}	1.1 ^{abc}
P9	1.3 ^e	1.1 ^{AB}	1.1 ^{AB}	1.1 ^{AB}	1.0 ^A	0.9 ^A	3.5 [*]	1.1 ^{bc}
P10	1.0 ^{ab}	1.1	1.1	1.0	1.0	0.9	0.9 ^{ns}	1.0 ^{abc}
F	6.4 ^{***}	0.9 ^{ns}	0.4 ^{ns}	0.5 ^{ns}	0.4 ^{ns}	0.6 ^{ns}		1.9 [*]
MEAN	1.1 ^D	1.1 ^{CD}	1.0 ^{BC}	1.0 ^B	1.0 ^B	0.9 ^A	13.6 ^{***}	

The application doses were statistically significant with respect to RadT in all populations, except in P2, P6, and P9. At the same time, the population-based changes in RadT were not statistically significant at all doses, except the control group. Generally, in all populations, the lowest RadT value was obtained at the 1000 mg/L dose of nanoparticle application, while the highest value was obtained in the control group. The RadT value was observed to be decreasing as the nanoparticle concentration increased.

On a population basis, while the lowest value was generally obtained in P7, the highest value was obtained in P4. In the control group, the lowest value was obtained in P7 (1.0%), while the highest value was obtained in P9 (1.3%).

Table 8. Changes in RadL Depending on Population and Nanoparticle Application

	Control	200	400	600	800	1000	F	MEAN (Nano1)
P1	5.8	4.7 ^B	4.5 ^B	4.2 ^{abAB}	4.3 ^{abcAB}	3.6 ^A	7.9 ^{***}	4.5 ^{bc}
P2	5.3	4.9	4.9	4.5 ^{ab}	4.4 ^{abc}	4.1	1.2 ^{ns}	4.7 ^{cd}
P3	5.6	3.9 ^A	3.9 ^A	3.6 ^{aA}	3.4 ^{abA}	3.3 ^A	3.7 [*]	3.9 ^a
P4	5.2	5.1	5.0	5.0 ^b	4.6 ^c	4.4	0.8 ^{ns}	4.9 ^{cd}
P5	5.7	4.7 ^{AB}	4.5 ^{AB}	4.2 ^{abA}	4.3 ^{abcA}	3.5 ^A	3.2 [*]	4.5 ^{bc}
P6	5.3	4.7	4.6	4.3 ^{ab}	4.2 ^{abc}	4.1	1.7 ^{ns}	4.5 ^{bc}
P7	5.4	3.8 ^A	3.7 ^A	3.4 ^{aA}	3.3 ^{aA}	3.2 ^A	7.2 ^{***}	3.8 ^a
P8	5.2	5.0	4.9	4.5 ^{ab}	4.4 ^{bc}	4.2	1.1 ^{ns}	4.7 ^{cd}
P9	5.6	5.2	5.2	4.9 ^b	4.8 ^c	4.7	1.9 ^{ns}	5.1 ^d
P10	5.2	4.3 ^{AB}	4.2 ^{AB}	3.9 ^{abA}	3.7 ^{abcA}	3.4 ^A	3.2 [*]	4.1 ^{ab}
F	0.4 ^{ns}	2.0 ^{ns}	2.0 ^{ns}	2.3 [*]	2.2 [*]	1.7 ^{ns}		6.0 ^{***}
MEAN	5.4 ^E	4.7 ^D	4.6 ^{CD}	4.2 ^{BC}	4.1 ^{AB}	3.8 ^A	21.6 ^{***}	

Taking the results in the table into consideration, it was found that the application doses were not statistically significant in all populations, except in P1, P3, P5, P7, and P10 with respect to RadL. At the same time, the population-based changes in RadL were not statistically significant at all doses, except at 600 and 800 mg/L dose of nanoparticle applications. In general, while the lowest RadL value in all populations was obtained at the 1000 mg/L dose of nanoparticle application, the highest value was obtained in the control group. It was observed that the RadL value decreased as the nanoparticle concentration increased. On a population basis, the lowest value was generally obtained in P7, while the highest value was obtained in P9.

The Ag nanoparticles negatively affected germination and seedling parameters in oriental beech seeds. This impact varied, depending on parameters. The germination rate was observed to be decreasing compared to the control group even at the lowest dose of 20 mg/L, and there was a significant decrease in seedling characters starting from the 60 mg/L dose. This situation shows that the beech forests were significantly affected by Ag nanoparticle pollution, especially in the early period.

Silver nanoparticles are extensively used in many areas, and there are many studies aiming to identify the effects of these nanoparticles on plant development. These studies generally revealed that Ag nanoparticles positively affected germination parameters in the early stages. As a result of the study conducted by Salih *et al.* (2022), nanoparticles containing Ag had a significant effect on the germination rate, germination rate index, and development of stem and root systems of tomato seeds. The plants exposed to silver nanoparticles showed both positive and negative effects. Also, it is stated that silver nanoparticles stimulate the germination of seeds of edible plants, but negatively affect the growth of vegetables and reduce their mass (Siddiqi and Husen 2022).

This result has been supported by many other studies conducted on the subject. In *Phaseolus vulgaris* and *Zea mays* plants, it was found that low concentrations of silver nanoparticles had a stimulating effect on the growth of plants, whereas the increased concentrations had an interfering effect. It was also found that increasing the silver nanoparticle concentration from 20 ppm to 60 ppm led to an increase in the lengths of shoot and root, leaf surface area, chlorophyll, carbohydrate and protein contents of these plants, yet this effect was reversed with increasing concentrations (Salama 2012). It was also found that Ag nanoparticles could cause a decrease in germination of wetland plants, and that root growth would be affected by Ag exposure much more than leaf growth, in general (Yin *et al.* 2012). It was also stated that silver nanoparticles inhibit seed germination at low concentrations in *Linum usitatissimum*, *Lolium perenne*, and *Hordeum vulgare* plants (El-Temsah and Joner 2012). Moreover, small-sized Ag nanoparticles (10 and 20 nm in size) are stated to be reducing the growth of *Triticum aestivum* seedling roots (Lahuta *et al.* 2022a). Silver nanoparticles are beneficial during bean seed germination period, but negatively affect plant development in the latter stages (Pražak *et al.* 2020).

In a study conducted on grains, the samples were found to be germinating well, but afterwards the growth of the roots was found to be gradually slowing down. The hazardous effect of Ag nanoparticles on roots was manifested as a shortening and thickening of roots, browning of root tips, death of epidermal cells, progression from the apical meristem to the root hair, and inhibition of root hair development. Sucrose, maltose, phosphoric acid, and some amino acids were accumulated in the roots. In contrast to the roots, the concentration of most metabolites decreased in the coleoptile and endosperm. Changes in the concentration of polar metabolites of seedlings revealed the effects on the primary metabolism, defects in the mobilization of storage materials, and the translocation of both

sugars and amino acids from endosperm to growing seedlings (Lahuta *et al.* 2022b). These results suggest that despite the positive effects of Ag nanoparticles, they mainly have negative effects on plant development during germination or the subsequent period, and that they definitely have negative effects at high doses.

Many national and international environmental organizations and institutions report that there have been unprecedented vital disturbances, diseases, natural degenerations, and sudden losses occurring in natural resources due to nanoparticle pollution, which has reached higher levels, especially in underdeveloped or developing countries. According to the latest reports published within this context, it is reported that the annual losses in plant, animal, and human populations due to nanoparticle pollution will reach up to 33% within the next 20 to 40 years, and new virological elements, which have never been seen before, will form (Lahiani *et al.* 2015). Within this scope, plants are the leading group to be most affected by nanoparticle pollution. Plants, which are relatively weaker than other living elements and populations in terms of defence mechanisms and have very high possibilities of being used as intermediate hosts, serve to create very suitable conditions for interaction within nanoparticles and get damaged significantly, both at the individual and social levels (Ozdemir 2023).

Expectations for the level of damage caused by the nanoparticle pollution to increase in the near future due to the unexpected harmful interactions of nanoparticle pollution, especially global warming, and the signals received from nature regarding these expectations, are increasing rapidly. Negative and unexpected events occurring, especially in plants and their natural life processes, are associated with environmental problems. Different types of environmental pollution are very effective in this regard (Savas *et al.* 2022; Istanbulu *et al.* 2023; Koc *et al.* 2023). In the studies conducted, it is presented that nanoparticles weaken germination considerably, even in the seeds of short-lived species with very rapid germination ability and energy, and that it stops completely after reaching a certain toxicological threshold value. Low doses of nanoparticles can be tolerated in some xerophyte plant species. However, toxic effects are observed in these plants after a certain level (Krishnaraj *et al.* 2012; Mahakham *et al.* 2017). It is emphasized that the most important and destructive effect of global climate change will show itself in the form of temperature increase and drought (Cetin *et al.* 2023; Cobanoglu *et al.* 2023b; Isinkaralar *et al.* 2023). For this reason, it is estimated that the effects of drought stress will be felt to a greater extent due to nanoparticle effects in plants.

The germination and seedling parameters in seeds collected from different populations varied to a considerable extent based on the populations. For instance, the P7 population was among the populations most affected by nanoparticles, while the P4 and P9 populations were among the least affected ones. All phenotypic characters of plants are formed under the interaction of genetic structure (Sevik *et al.* 2021; Kurz *et al.* 2023; Tandogan *et al.* 2023) and environmental factors (Özel *et al.* 2022; Erdem *et al.* 2023; Yigit *et al.* 2023). In addition to environmental factors, practices such as fertilization (Sadak and Bakhom 2022; Bakhom *et al.* 2023) and stress factors such as drought (Bakhom *et al.* 2022; Sadak *et al.* 2022; Hanafy and Sadak 2023), UV-B, radiation, and heavy metals also affect plant growth (Ucun Özel *et al.* 2019; Özel *et al.* 2021a,b; Ghoma *et al.* 2023). The different levels of genetic structure and genetic diversity cause their reactions to environmental stress factors to be different. It is emphasized that the phytotoxicity of silver nanoparticles on the seed germination and seedling development of plants depends on the properties and concentration of nanoparticles, as well as the plant species and their level of stress tolerance (Lahuta *et al.* 2022a).

There have not been enough studies on the effects of nanoparticles on ecosystems and forests yet. In future studies, the level of increase in the concentrations of nanoparticles in nature could be investigated. In addition, studies can focus on the minimum concentrations at which various kinds of nanoparticles pose a danger. The effects of nanoparticles on species and ecosystems should be investigated in multiple ways. For example, how nanoparticles affect fruit yield and quality, how they affect species and thus biodiversity, and what their effects are on fauna and microfauna should be investigated in detail.

CONCLUSIONS

1. Even though some research has been conducted on the effects of nanoparticles on the seeds and germination processes of some forest trees, especially in Europe and the Americas, these are obviously not sufficient, considering the tree species, types, and reproductive processes around the world.
2. In countries that are rich in terms of forest resources, as in Türkiye, and where these resources have a substantial impact on the country's economy, the effects of forest trees, their seeds, germination, development, and other biological functions should be examined through comprehensive research.
3. The decrease in germination percentage will have greater effects in species such as beech, which is discussed in this study and whose abundant seed year intervals are quite long, and studies on these species should be prioritised. Moreover, the study results suggest that some populations are less affected by pollution. These populations can be used as main seed sources for seedling production.

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