# Effects of Nanoparticle Applications on Seedling Survival and Morphological Characteristics in Scots Pine Afforestation

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This study was conducted in the afforestation area, using bare-root 2+0-year-old Scots pine seedlings from Kastamonu. The study aimed to determine the impact of nanoparticle (NP) applications on seedling morphological characteristics and seedling survival success. Three different concentrations (low, medium, high) and four different nanoparticle types [$Fe_3O_4$, CuO, ZnO, $TiO_2$] were applied to the plant root-dipping method in the study. The effects of NP treatments on seedling height (SH), root collar diameter (RCD), stem fresh weight (SFW), root new weight (RFW), seedling fresh weight (SEFW), root dry weight (RDW), stem dry weight (SDW), seedling dry weight (SEDW), sturdiness quotient (SI), root: shoot ratio (*R*/*S*), and seedling survival in the field were evaluated. The study results revealed that NP types significantly affected all seedling variables except RFW, SDW, RDW, and SEDW, and NP doses significantly affected all seedling variables except RFW. The binary interaction effects of NP types and doses had a significant effect on all seedling variables, and higher values were obtained compared to the control treatment. Medium and high NP doses were more effective in seedling growth than low doses; the percentage of seedling survival was 61.4% in the control treatment and 95% in the $TiO_2$-Medium NP treatment combination.

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## INTRODUCTION

Nanomaterials are structures with a size between 1 and 100 nm (Nel *et al.* 2006), and their strong physical and chemical properties enable them to be used in many fields (Rao *et al.* 2005; Gürmen and Ebin 2008; Ayan *et al.* 2021). These materials can be present in nature, and their concentrations are steadily increasing due to nanotechnological developments (Miller *et al.* 2004). People utilize metal-based nanoparticles (NPs) to make life easier across different sectors, and their use has rapidly grown in the last decade. Currently, the most popular NP structures are silver (Ag), as well as oxides of titanium (Ti), zinc (Zn), aluminum (Al), nickel (Ni), gold (Au), indium (In), molybdenum (Mo), copper (Cu), iron (Fe), bismuth (Bi), silica (Si), cobalt (Co), and tin (Sn). The most widely manufactured and commercially utilized metal-oxide NPs are titanium dioxide ($TiO_2$, zinc oxide (ZnO), iron oxide ($Fe_3O_4$), copper oxide (CuO), silicon dioxide ($SiO_2$), aluminum oxide ($Al_2O_3$), cerium dioxide ($CeO_2$), magnesium oxide (MgO), cuprous oxide ($Cu_2O$), nickel oxide (NiO), lanthanum oxide ($La_2O_3$), zirconium dioxide ($ZrO_2$), and indium oxide ($In_2O_3$) (Rajput *et al.* 2018). These materials have ecotoxicological effects, can accumulate in biological systems, and can be menacing when they undergo bioaccumulation and biodegradation in the food chain (Kuzma 2008). Moreover, nanoparticles (NPs) can cause different environmental impacts due to their physicochemical properties (Ma and Wang 2010). They have found wide application possibilities, particularly in medicine, pharmacy, construction, cosmetics, optics, and electronics sectors (Ruffini and Cremonini 2009; Kaweeteerawat *et al.* 2015; Tunca 2015).

In recent years, the effects of NP applications and how they are transported on plants have gained increasing importance in plant research (Du *et al.* 2011; Kundu *et al.* 2015). However, despite size exclusion limitations of 20 nm (Ma and Yan 2018; Ballikaya *et al.* 2022), it is commonly believed that particles > 100 nm can be taken up by plant roots. The results on root uptake vary depending on the kind of NPs, plant characteristics, and length of exposure (Ma and Wang 2010). According to Lv *et al.* (2019), stomata appear to be the main pathway for the foliar uptake of a wide variety of NPs. According to Avellan *et al.* (2021), the plant shape and physiological state (between 40 nm and 1 m) have an impact on the stomatal uptake of NPs. Nanoparticles promote plant growth by facilitating the uptake of nutrients from the soil because of their high surface volume and electronic structure, are used as fertilizers by increasing disease resistance, and are used in studies to prevent pests and diseases that infect plants (Servin *et al.* 2015). It is well known in this context that silver nanoparticles are extremely effective against a wide variety of fungi (Xue *et al.* 2016), bacteria (Rai *et al.* 2012), and viruses (Ardestani *et al.* 2015), including phytopathogenic ones. However, although silver nanoparticles (AgNP) in soil pose a significant environmental risk, there is little research on how such contamination may affect ectomycorrhizal fungi (EMFs) (Sweet and Singleton 2015).

Most studies suggest that NPs of 5 nm in size are taken up by both roots and aerial parts of plants and are transported through both the phloem and xylem (Dietz and Herth 2011; Wang *et al.* 2016; Ruttkay-Nedecky *et al.* 2017; Li *et al.* 2018). The NP applications, which are getting increasingly important, especially in agricultural studies, are being investigated for NP types and doses that will support breeding studies and ensure rapid germination and growth of seeds (Azura *et al.* 2017). The positive effects of pre-sowing treatment of wheat, maize, and rapeseed seeds with Cu and Zn NPs have been reported, but excess copper is toxic, and the root barrier plays an important role in the formation of tolerance to the surplus of this metal (Lebedev *et al.* 2016; Yausheva *et al.* 2017).

Moreover, the use of nanoparticles in forestry has become increasingly important in recent years. According to certain studies, mycorrhizal colonization of plant roots is negatively impacted (Dubchak *et al.* 2010), while other studies (Judy *et al.* 2015; Cao *et al.* 2017) contend that low nanoparticle concentrations have no effect on mycorrhization levels but that high concentrations have detrimental effects. Contrastingly, Feng *et al.* (2013) showed that AgNPs have a stimulatory effect on the development of arbuscular mycorrhiza, regardless of the used doses. According to Sweet and Singleton (2015), the variety of ectomycorrhizal fungi in the roots of *Pinus muricata* seedlings may drastically decrease as a result of soil contamination by AgNPs. Olchowik *et al.* (2017) found varying effects of metal nanoparticles on the type and concentration of ectomycorrhizae that form in pedunculate oak seedlings. Copper nanoparticles (Cu-NPs) were discovered to have a stimulatory effect at low concentrations but an inhibitory influence at high concentrations.

Ag-NPs encouraged the formation regardless of concentration. In addition to the tree species, growth conditions, substrate type (soil or various nutrient media), temperature, and light intensity, their impact also appears to be influenced by the dosage, application method (foliar or soil), and concentration of the nanoparticles (Ruffini and Cremonini 2009). Zakharova *et al.* (2019) looked into how white poplar, crack willow, hairy birch, red oak, and pine micro clones adapted to *ex vitro* settings when exposed to zirconium trisulphide nanoplates and silver nanoparticles. The findings suggest that zirconium trisulphide nanoplates and silver nanoparticles have great potential for safeguarding woody species microclones when they are being transferred to non-sterile glasshouse environments. Additionally, because plants are known to be sensitive to nanoparticles, it is feasible that the presence of NP could have a direct impact on the growth of the trees (Yin *et al.* 2012). In addition, given the changes in climate, NP applications should be investigated to increase the seedling survival rate and to ensure better quality root and stem development in afforestation studies conducted in arid and semi-arid areas and that NP application can support forest tree breeding studies. Indeed, it is important for plants to benefit more from the soil in arid and semi-arid regions. For this purpose, using NPs in applications that increase drought resistance can be useful (Ashkavand *et al.* 2015). Reforestation studies for breeding trees resistant to ecological conditions in arid and semi-arid regions have become important to investigate the effectiveness of nanoparticles, especially in recent years when the effects of climate change have been intense. To this end, Ashkavand *et al.* (2015) suggested that nano silicon application on *Crataegus* sp. may be advantageous in reducing the harmful effects of drought stress. In Turkey, the development of seedlings was investigated by treating Anatolian black pine (*Pinus nigra* Arnold.) (Çelikbaş 2019), which is mainly used in the afforestation of semi-arid areas, Scots pine (*Pinus sylvestris* L.) (Çelikbaş 2019) and red pine (*Pinus brutia* Ten.) (Ayan *et al.* 2021) seeds with different NP types and doses before sowing, and the development of seedlings was investigated. It was reported that NP application was generally beneficial for black pine and Scots pine seeds germinated under laboratory conditions but did not contribute positively to red pine seeds sown under field conditions. Thus far, most of the research on the effect of nanoparticles on plants has been conducted in hydroponic culture (Bernhardt *et al.* 2010) or on peat-based substrates used in nursery production (Olchowik *et al.* 2017). Our hypothesis in this study is to examine the positive or negative effects of nanoparticle types and doses applied to the root area of saplings before planting on the development of saplings.

This study attempted to reveal the effects of applying different doses and types of nanoparticle solutions to the roots of seedlings before planting under field conditions on seedling survival rate and morphological development of seedlings in the field.

## EXPERIMENTAL

In this research, NPs were added to the root zone of bare-rooted Scots pine seedlings. The effects of four different types of NP solutions at different dosage levels on seedling growth under field conditions were investigated using the root dipping method.

### Materials

The bare-root 2+0 aged (2-year-old seedling produced by generative production method under nursery conditions) Scots pine seedlings originating from Araç-Dereyayla, Kastamonu were obtained from Kastamonu Taşköprü Forest Nursery. The experiment was established in section 191 of İhsangazi Forest Management Directorate, Mergüze Forest Management Chiefdom within the boundaries of Kapaklı Village (Coordinate: X 53° 89'50'' - Y 45°53'13''.1 / 41°7'33''.91 N - 33°27'52''.97 E), which is 52.4 ha in size (Fig. 1).

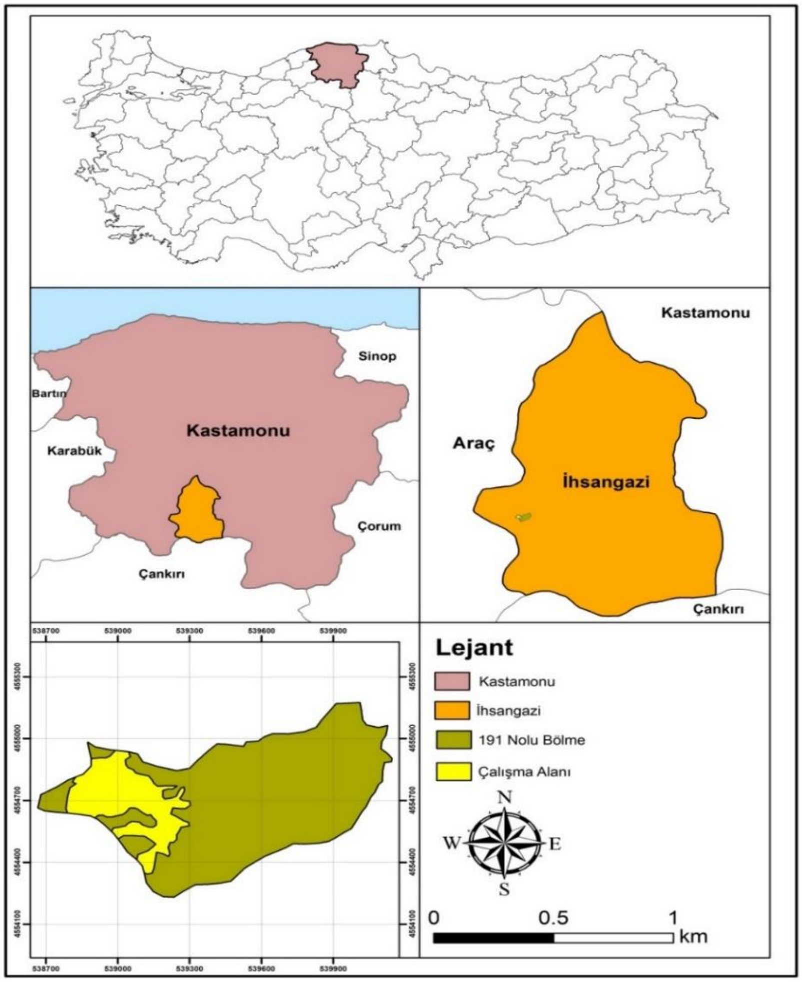


**Fig. 1.** Study area

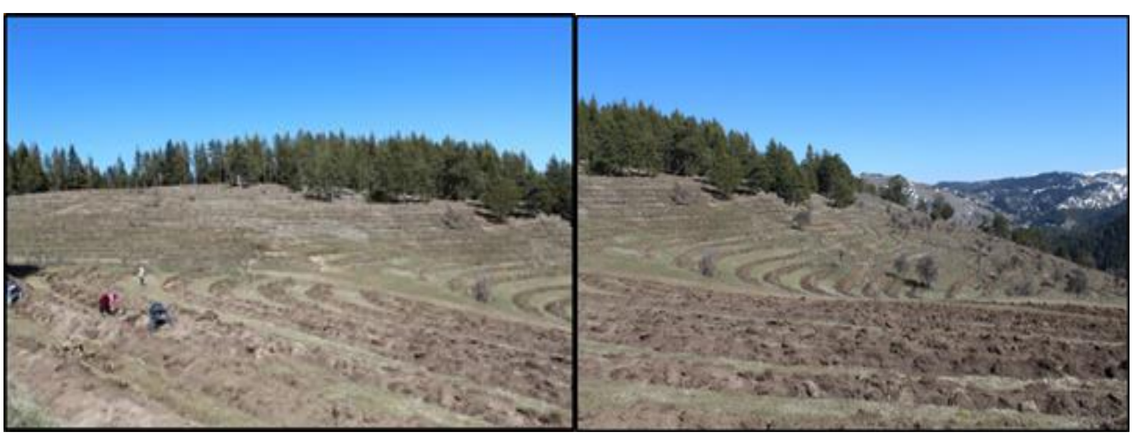

**Fig. 2.** General view of the research area

The site has an altitude of 1579 m with a southern facing and a slope of 31 to 60%. The average annual temperature of the region is 9.8 °C and the average rainfall is 482.3 mm. The soil structure of the site, which has sedimentary bedrock, is sandy-clay, and the seedlings were planted in the terraces established by edge planting at 1.5 × 1.5 $m^2$ intervals in April of 2019 (Fig. 2).

## Methods

The doses specified in Table 1 were used in this study for the solutions of the most widely used NP types (Ashkavand *et al.* 2015; Rajput *et al.* 2018; Aleksandrowicz-Trzcińska *et al.* 2019; Khan *et al.* 2021).

Except for the control treatment, 30 seedlings were used for each NP solution and each different dose, and the experiment was established in a randomized block design with three replications. Using the root dipping method, the root zones of the seedlings were pretreated with NP solutions for 30 min before planting.

To determine the morphological characteristics of the seedlings in the dormant stage outside the vegetation period, the seedlings from the control treatment and each NP treatment group were uprooted in November 2019 without damaging the roots. Before uprooting, the root collar diameter of the seedlings was measured with a digital caliper with a precision of 0.1 mm, and the seedlings' heights were measured with a steel tape measure with a precision of 1 mm. The roots of the seedlings were cleaned from the soil and individually tagged for determination of root/stem ratio and seedling fresh and dry weights. They were transferred to the laboratory for analysis.

**Table 1.** Types and Doses of Used Nanoparticles

| NP Names | NP Levels | NP Doses (mg/L) |
|---|---|---|
| ZnO | High | 2000 |
| | Medium | 1200 |
| | Low | 400 |
| $Fe_3O_4$ | High | 2000 |
| | Medium | 1200 |
| | Low | 400 |
| $TiO_2$ | High | 1000 |
| | Medium | 600 |
| | Low | 400 |
| CuO | High | 1000 |
| | Medium | 600 |
| | Low | 400 |

*Measurements and calculations made in the laboratory*

In the seedlings brought to the laboratory in November to December 2019, seedling root collar diameter (RCD-mm) was determined with 0.1 mm precision from the root collar, seedling height (SH-cm) was determined with 0.1 cm precision from the root collar diameter to the terminal shoot tip, and the sturdiness index (SI) was calculated as the ratio of SH to RCD (Aphalo and Rikala 2003) as given below in Eq. 1:

$$Sturdiness\ index\ (SI) = \frac{Seedling\ height\ (mm)}{Root\ collar\ diameter\ (mm)} \quad (1)$$

The parts of the seedlings above and below the RCD were measured with a precision of 0.001 g. The weight values were stem fresh weight (SFW-g) and root fresh weight (RFW-g), the sum of these two values was seedling fresh weight (SEFW-g), while stem dry weight (SDW-g), root dry weight (RDW-g), and seedling dry weight (SEDW-g) were determined by drying these parts at 101 +/- 4 °C for 24 h. The root:shoot ratio (*R*/*S*) index was calculated as the ratio of stem dry weight to root dry weight (Ayan 2002), given as Eq. 2:

$$Root{:}\,Shoot\ ratio\ (R/S) = \frac{Stem\ Dry\ Weight\ (g)}{Root\ Dry\ Weight\ (g)} \tag{2}$$

### Statistical Evaluations

Multivariate analysis of variance was applied to determine whether RCD, SH, SFW, RFW, SEFW, SDW, RDW, SEDW, SI, and *R*/*S* variables of seedlings showed significant differences in terms of various NP treatments and different doses, and homogeneous groups were determined by Duncan test. All data were analyzed with IBM SPSS 23 software (IBM Corp., Armonk, NY, USA) at a p = 0.05 significance level.

## RESULTS AND DISCUSSION

The significant effects of NP types applied to Scots pine seedlings on all other variables except RFW, SDW, RDW, and SEDW variables are shown in Table 2. According to the general average values given in Table 2, the lowest values were in the control group in all studied seedling variables. While the $TiO_2$ NP solution was the most efficient in SH development, the lowest SH development in NP-treated seedlings was the ZnO solution. In terms of root collar diameter development, CuO, and $TiO_2$ were the most effective, while ZnO had the lowest effect. CuO NP application had the most positive effect on SFW, SDW, and SEFW variables. In terms of SI and R/S, it was determined that the seedlings allocated as control were more advantageous (Table 2).

The NP doses had a significant effect on all seedling variables except RFW (Table 3). Compared to the control treatment, all NP dose treatments had a positive effect on seedling fresh and dry weight values except SI and R/S. Among the doses, "Medium" and "High" amounts were more efficient than low doses (Table 3).

The highest values on the SH variable were detected in $TiO_2$-Medium (21.42 ± 0.93 cm) and CuO-High (21.38 ± 1.05 cm) dose applications. The effects of the same nanoparticles were also found to be high on the RCD [$TiO_2$-Medium (6.83 ± 0.27 mm), CuO-High (6.83 ± 0.32 mm)] variable. When Table 4 is examined, the ZnO-Medium treatment combination on the following variables [SFW (17.42 ± 4.34 g), RFW (6.06 ± 1.49 g), SEFW (23.47 ± 5.82 g), SDW (7.75 ± 1.98 g), RDW (7.75 ± 1.97 g) and SEDW (10.69 ± 2.71 g)] supported plant growth in the most positive way. The control treatment (20.01 ± 0.09) had the most favorable value in the SI variable, and the $TiO_2$-Low (2.15 ± 0.11) treatment combination had the best value in the R/S variable (Table 4).

It was determined that NP treatments generally had a positive effect on the survival rate of seedlings compared to the control treatment (61.39%), and the highest survival rate was obtained with $TiO_2$ NP (95%) applied at a medium dose (Table 5).

**Table 2.** Analysis of Variance and Duncan Test Results for the Effect of NP Type

| | | Mean ($\bar{X}$) ± Standard Error ($S\bar{x}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Types of NP** | **SH (cm)** | **RCD (mm)** | **SFW (g)** | **RFW (g)** | **SEFW (g)** | **SDW (g)** | **RDW (g)** | **SEDW (g)** | **SI** | **R/S** |
| Control | 14.05 ± 0.95d | 4.42 ± 0.29c | 9.59 ± 0.49b | 4.27 ± 0.23 | 13.86 ± 0.67b | 4.43 ± 0.23 | 2.18 ± 0.11 | 6.62 ± 0.32 | 20.17 ± 0.14c | 2.22 ± 0.08b |
| $TiO_2$ | 20.12 ± 0.70a | 6.09 ± 0.20a | 11.80 ± 0.77ab | 4.60 ± 0.22 | 16.40 ± 0.95ab | 5.32 ± 0.33 | 2.37 ± 0.10 | 7.70 ± 0.42 | 20.86 ± 0.10a | 2.23 ± 0.08b |
| ZnO | 16.37 ± 0.79c | 5.12 ± 0.24b | 12.81 ± 1.63a | 4.88 ± 0.55 | 17.69 ± 2.16a | 5.80 ± 0.73 | 2.46 ± 0.28 | 8.26 ± 0.99 | 20.41 ± 0.11bc | 2.61 ± 0.20a |
| $Fe_3O_4$ | 17.74 ± 0.72bc | 5.43 ± 0.21b | 12.23 ± 0.77a | 4.37 ± 0.28 | 16.60 ± 1.02ab | 5.59 ± 0.35 | 2.28 ± 0.14 | 7.88 ± 0.48 | 20.70 ± 0.11ab | 2.50 ± 0.08ab |
| CuO | 19.48 ± 0.63ab | 6.44 ± 0.19a | 13.38 ± 0.74a | 4.80 ± 0.26 | 18.18 ± 0.96a | 5.91 ± 0.47 | 2.29 ± 0.17 | 8.20 ± 0.62 | 20.73 ± 0.08ab | 2.69 ± 0.15a |
| F-value | *9.43* | *11.26* | *3.49* | *0.75* | *2.63* | *2.61* | *0.443* | *1.82* | *5.79* | *3.32* |
| P-level | *0.00* | *0.00* | *0.01* | *0.56* | *0.03* | *0.36* | *0.77* | *0.13* | *0.00* | *0.01* |

**Table 3.** Analysis of Variance and Duncan Test Results for the Effect of NP Dose

| | | Mean ($\bar{X}$) ± Standard Error ($S\bar{x}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Doses of NP** | **SH (cm)** | **RCD (mm)** | **SFW (g)** | **RFW (g)** | **SEFW (g)** | **SDW (g)** | **RDW (g)** | **SEDW (g)** | **SI** | **R/S** |
| Control | 14.05 ± 0.95b | 4.42 ± 0.29b | 9.59 ± 0.49c | 4.27 ± 0.23 | 13.86 ± 0.67b | 4.43 ± 0.23 c | 4.43 ± 0.22 c | 6.62 ± 0.32 b | 20.17 ± 0.14b | 2.22 ± 0.08 b |
| High | 19.41 ± 0.59a | 6.06 ± 0.18a | 12.35 ± 0.75ab | 4.55 ± 0.22 | 16.90 ± 0.93 ab | 5.53 ± 0.35b | 5.53 ± 0.34 b | 7.90 ± 0.45ab | 20.77 ± 0.08a | 2.46 ± 0.13ab |
| Medium | 18.30 ± 0.63a | 5.73 ± 0.20a | 14.35 ± 1.22a | 5.15 ± 0.42 | 19.50 ± 1.63a | 6.57 ± 0.6a | 6.56 ± 0.59 a | 9.15 ± 0.81 a | 20.67 ± 0.09a | 2.63 ± 0.11a |
| Low | 17.57 ± 0.64a | 5.52 ± 0.19a | 10.96 ± 0.58bc | 4.29 ± 0.2 | 15.25 ± 1.78bc | 4.86 ± 0.23bc | 4.86 ± 0.23 bc | 6.99 ± 0.32b | 20.59 ± 0.09a | 2.44 ± 0.11ab |
| F-value | *8.38* | *8.54* | *7.22* | *2.04* | *5.81* | *6.67* | *6.67* | *5.24* | *5.02* | *2.60* |
| P-level | *0.00* | *0.00* | *0.00* | *0.11* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* | *0.045* |

**Table 4.** Analysis of Variance and Duncan Test Results for the Binary Interactions of NP Type and Dose Factors

| | **Mean ($\bar{X}$) ± Standard Error ($S\bar{x}$)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Types and Doses of NP** | **SH (cm)** | **RCD (mm)** | **SFW (g)** | **RFW (g)** | **SEFW (g)** | **SDW (g)** | **RDW (g)** | **SEDW (g)** | **SI** | **R/S** |
| Control | 13.27 ± 0.59 e | 4.10 ± 0.18 e | 9.59 ± 0.49 C | 4.27 ± 0.23 ab | 13.86 ± 0.67 bc | 4.43 ± 0.23 c | 4.43 ± 0.22 c | 6.62 ± 0.32 bc | 20.1 ± 0.88 c | 2.22 ± 0.08 ab |
| $TiO_2$-High | 20.93 ± 1.11 ab | 6.20 ± 0.32 ab | 12.61 ± 1.42 abc | 4.67 ± 0.36 ab | 17.28 ± 1.73 abc | 5.56 ± 0.63 abc | 5.55 ± 0.62 abc | 8.06 ± 0.76 abc | 30.22 ± 1.64 a | 2.23 ± 0.15 ab |
| $TiO_2$-Medium | 21.42 ± 0.93 a | 6.83 ± 0.27 a | 12.39 ± 1.53 bc | 5.08 ± 0.46 ab | 17.47 ± 1.90 abc | 5.64 ± 0.67 abc | 5.63 ± 0.67 abc | 8.06 ± 0.87 abc | 30.07 ± 1.18 a | 2.31 ± 0.14 a |
| $TiO_2$-Low | 18.02 ± 1.52 abcd | 5.23 ± 0.43 bcde | 10.39 ± 0.97 bc | 4.06 ± 0.32 b | 14.44 ± 1.23 bc | 4.78 ± 0.42 bc | 4.77 ± 0.41 bc | 7.00 ± 0.54 bc | 25.55 ± 2.15 abc | 2.15 ± 0.11 b |
| ZnO-High | 17.00 ± 1.31 bcde | 5.30 ± 0.40 bcd | 11.78 ± 1.68 bc | 4.78 ± 0.58 ab | 16.56 ± 2.17 bc | 5.31 ± 0.73 bc | 5.30 ± 0.72 bc | 7.81 ± 0.97 abc | 24.84 ± 1.87 abc | 2.48 ± 0.46 ab |
| ZnO-Medium | 15.83 ± 1.54 de | 4.84 ± 0.47 cde | 17.42 ± 4.34 a | 6.06 ± 1.49 a | 23.47 ± 5.82 a | 7.75 ± 1.98 a | 7.75 ± 1.97 a | 10.69 ± 2.71 a | 22.77 ± 2.17 bc | 2.69 ± 0.17 ab |
| ZnO-Low | 16.29 ± 1.26 cde | 5.22 ± 0.38 bcde | 9.22 ± 1.04 c | 3.81 ± 0.41 b | 13.03 ± 1.34 c | 4.33 ± 0.42 c | 4.33 ± 0.42 c | 6.28 ± 0.56 c | 24.75 ± 1.90 abc | 2.65 ± 0.36 ab |
| $Fe_3O_4$-High | 18.33 ± 1.19 abcd | 5.90 ± 0.37 abc | 12.03 ± 1.54 bc | 4.19 ± 0.40 ab | 16.22 ± 1.89 bc | 5.50 ± 0.71 abc | 5.5 ± 0.71 abc | 7.69 ± 0.88 abc | 26.46 ± 1.76 ab | 2.48 ± 0.16 ab |
| $Fe_3O_4$-Medium | 15.65 ± 1.36 de | 4.58 ± 0.39 de | 12.67 ± 1.23 abc | 4.33 ± 0.50 ab | 17.00 ± 1.69 abc | 5.81 ± 0.58 abc | 5.8 ± 0.57 abc | 8.17 ± 0.82 abc | 25.50 ± 2.20 abc | 2.56 ± 0.12 ab |
| $Fe_3O_4$-Low | 19.23 ± 1.12 abcd | 5.79 ± 0.32 abc | 12.00 ± 1.30 bc | 5.48 ± 0.56 ab | 16.58 ± 1.82 bc | 5.47 ± 0.58 abc | 5.47 ± 0.57 abc | 7.78 ± 0.83 abc | 29.11 ± 1.68 a | 2.47 ± 0.15 ab |
| CuO-High | 21.38 ± 1.05 a | 6.83 ± 0.32 a | 12.97 ± 1.46 abc | 4.56 ± 0.37 ab | 17.53 ± 1.76 abc | 5.78 ± 0.77 abc | 5.77 ± 0.76 abc | 8.03 ± 1.05 abc | 29.11 ± 1.37 a | 2.63 ± 0.19 ab |
| CuO-Medium | 20.33 ± 0.96 abc | 6.68 ± 0.32 a | 14.94 ± 1.12 ab | 5.11 ± 0.47 ab | 20.06 ± 1.53 ab | 7.08 ± 1.05 ab | 7.08 ± 1.04 ab | 9.67 ± 1.37 ab | 28.33 ± 1.29 ab | 2.95 ± 0.36 a |
| CuO-Low | 16.73 ± 1.17 cde | 5.82 ± 0.35 abc | 12.22 ± 1.23 bc | 4.72 ± 0.53 ab | 16.94 ± 1.72 abc | 4.86 ± 0.44 bc | 4.86 ± 0.43 bc | 6.92 ± 0.59 bc | 24.34 ± 1.57 abc | 2.49 ± 0.21 ab |
| F-value | *7.76* | *9.45* | *2.55* | *1.04* | *2.14* | *2.21* | *2.21* | *1.77* | *5.605* | *1.34* |
| P-value | *0.00* | *0.00* | *0.00* | *0.041* | *0.02* | *0.01* | *0.01* | *0.04* | *0.00* | *0.02* |

**Table 5.** Statistics on the Interactive Effect of Nanoparticle Type and Dose on Seedling Survival

| NP Types/Doses | Total Seedling Number | Seedling of Drying Number | Seedling of Survival Number | Percentage (%) |
|---|---|---|---|---|
| Control | 360 | 139 | 221 | 61.39 |
| $TiO_2$-High | 60 | 7 | 53 | 88.33 |
| $TiO_2$-Medium | 60 | 3 | 57 | 95.00 |
| $TiO_2$-Low | 60 | 16 | 44 | 73.33 |
| ZnO-High | 60 | 14 | 46 | 76.67 |
| ZnO-Medium | 60 | 19 | 41 | 68.33 |
| ZnO-Low | 60 | 13 | 47 | 78.33 |
| $Fe_3O_4$-High | 60 | 10 | 50 | 83.33 |
| $Fe_3O_4$-Medium | 60 | 16 | 44 | 73.33 |
| $Fe_3O_4$-Low | 60 | 8 | 52 | 86.67 |
| CuO-High | 60 | 5 | 55 | 91.67 |
| CuO-Medium | 60 | 5 | 55 | 91.67 |
| CuO-Low | 60 | 9 | 51 | 85.00 |

When studies on various plant species are examined, it is apparent that the effects of NP solutions applied in different types and doses vary considerably (Doğaroğlu and Köleli 2016; Ayan *et al.* 2021). In a study conducted by Sharma *et al.* (2012) on mustard plants, it was reported that the application dose of AgNPs was important and had a positive effect on the vitality indices of seedlings. Trees are able to successfully absorb Au-NPs and transport them through the plant system, according to Ballikaya *et al.* (2022) using species-specific modes of interaction linked to physical and physiological parameters. Through contrasting leaf-to-root and root-to-leaf paths, it was determined that two significant tree species, European beech (*Fagus sylvatica* L.) and Scots pine, are capable of absorbing and transporting differently charged Au-NPs into their stem. In both species, roots and leaves absorbed Au-NPs, and a tiny portion was also transferred to the stem. Au-NPs moved from leaves to roots but not the other way around. Askary *et al.* (2016) found that the application of $Fe_2O_3$ NP solution had a positive effect on seedling growth in mint plants, while Almutairi (2017) reported that NP application for cress plants promoted growth. Similarly, Vannini *et al.* (2013) reported that NP application increased root elongation in arugula plants. However, Zhu *et al.* (2008), in a study examining the effect of $Fe_3O_4$ NPs, found that zucchini plants grown in an aqueous medium containing NPs accumulated NPs, but they could not determine whether this accumulation had a positive or negative effect on the plant. Further, in Çalbay (2014) in the root tip cells of the onion plant, it was revealed that CuO NPs negatively affected growth. The effect of Ag-NPs and Cu-NPs on growth factors and spontaneous mycorrhizal colonization of roots in Scots pine seedlings raised in containers for two years was investigated. Nanoparticles were applied to leaves four times over the course of two growth seasons, at concentrations of 0, 5, 25, and 50 ppm. At all doses, the applied Cu-NPs promoted mycorrhizal colonization (Aleksandrowicz-Trzcinska *et al.* 2018). Fe-NPs and Cu-NPs solutions were applied to Scots pine seeds in a laboratory setting to increase their seeding properties and significantly lower the likelihood of mold damage to the seedlings (Polischuk *et al.* 2018). When pine seeds were germinating, the presence of Fe-NPs and Cu-NPs with sizes between 35 and 60 nm in a nutritional medium at concentrations between 2.10 and 4% and 2.10 to 2% significantly improved the germinating viability and energy. High concentrations prevent the availability of pine seeds because the increased amount of NPs (10%) lowers availability and germination energy (Polischuk *et al.* 2018). In this study, NPs applied in four different types ($TiO_2$, ZnO, $Fe_3O_4$, and CuO) positively affected seedling characteristics, such as SH, RCD, fresh, and dry (root, stem, and seedling) weights. In another study similar to this study, it was reported that high concentrations of $TiO_2$ NP treatment increased root growth of plants and $TiO_2$, positively contributed to root radicle elongation as the NP dose increased, and also positively affected the development of root collar diameters (Clement *et al.* 2013; Doğaroğlu and Köleli 2016). Similar results were found in this study in terms of SH development, and it was determined that $TiO_2$ had the highest effect on SH development and $TiO_2$-Medium treatment combination was the most effective. In this study, it was also demonstrated that "CuO-High", "CuO-Medium", and "$TiO_2$-Medium" NP treatments yielded good results in the development of RCD.

Researching the traits of plant growth when copper is added to the medium, however, is pertinent given the widespread usage of copper nanoparticles, particularly their application in the pre-sowing treatment of plant seeds. The growth characteristics of Scots pine during cultivation in a medium containing copper nanoparticles were examined in the current work. According to studies, growing pine in a media containing copper nanoparticles at concentrations of 0.025 to 0.1 M fully prevents the root system from developing. Growing the root in a medium with copper nanoparticles at a concentration of 6.25 mmol reduces the root's length to 27.9 mm and mass to 19.6 mg (Ryabinina *et al.* 2019). Raskar and Laware (2014) reported that ZnO NPs had positive effects on seed germination and root growth at low doses. In addition, a study on maize and rye plants reported that ZnO NPs inhibited the growth of plants at high concentrations of 2000 mg/L (Lin and Xing 2007). The majority of investigations have demonstrated that plants exposed to AgNP suffer negative growth effects. Depending on the plant species, growth circumstances (such as growing in soil or other nutrient media), and the amount and type of applied AgNP, the AgNP have distinct impacts on plants. According to Bayramzadeh *et al.* (2018), raising the concentration of Ag NP was demonstrated to have a substantial negative impact on Scots pine's early development characteristics when compared to controls for all planting dates. Silver nanoparticles in soil exhibited inhibitory effects on Scots pine seed germination and growth characteristics, and as time progressed, these inhibitory and harmful effects subsided (Bayramzadeh *et al.* 2018). The highest AgNP level considerably reduced pine seedlings' root length after just one month, which in turn had a negligible impact on the biomass of above-ground plants. However, both of the used AgNP levels significantly decreased the biomass of the pine roots and shoots after 4 months of growth (Sweet and Singleton 2015). According to research by Aleksandrowicz-Trzciska *et al.* (2019), the application of AgNPs causes Scots pine dry mass mycorrhizal colonization to rise at low concentrations of both 5 and 50 ppm. However, Scots pine and pedunculate oak seedlings that were one year old experienced some toxicity when exposed to AgNPs and CuNPs at greater doses of 25 and 50 ppm. Ayan *et al.* (2021) studied red pine seeds sown in the field according to the randomized blocks experimental design under field conditions. Eight different NP types (Silica, $TiO_2$, CuO, $Fe_2O_3$, $Fe_3O_4$, Au, Ag, and ZnO) and their five different dose levels were applied, and the effect of NP factor on SH, RCD, and FY of germinated seedlings was examined. It was determined that NP application had a negative effect on SH, RCD, and FY of germinated seedlings compared to the control group. Iron nanoparticles were shown to not affect the germination of seeds, but at the highest experimental dosage (100 mmol/L), they shortened the shoot and hindered the development of the root system (Kalyakina *et al.* 2019). In contrast to these studies, Çelikbaş (2019a, 2019b) observed that high doses (1200 mg/L and 2000 mg/L) of NP applications had positive effects on germination and growth in studies conducted on black pine and Scots pine seedlings. In this study conducted on Scots pine seedlings in the afforestation area, it was determined that seedling growth was positively influenced as the dose level of NP applications increased. Supporting the results of this study, Lin and Xing (2007) also emphasized that 2000 mg/L ZnO NP application promoted germination and root development. In this study, in this regard, it was determined that the seedlings with NP Type-Dose interactive application had thicker seedling RCD and higher SH and weight values compared to the control seedlings.

## CONCLUSIONS

1. In the present study, nanoparticle (NP) types and doses had significant effects on pine seedling morphological characteristics. Higher values were obtained compared to control seedlings.
2. The binary interaction effects of NP types and doses had a significant effect on all seedling variables, and higher values were obtained compared to the control treatment.
3. Medium and high NP doses were more effective in seedling development than low doses, and especially the positive effect of $TiO_2$-Medium dose NP treatment combination on seedling survival rate was determined.
4. These preliminary results may provide a basis for the use of NP applications in afforestation studies. Furthermore, the results of this study are valuable in determining the positive and negative effects of NPs and their application doses on the development of woody taxa and in providing ideas for further research on seedling production studies to determine whether NP application will affect the amount of carbon to be stored in the plants.

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### Declaration of Interests

The authors declare that they have no competing interests.

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