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Use of Nano-iron Fertiliser Additive Produced by Green Synthesis in Flame Tree (*Photinia frasserii*) and Smoke Tree (*Cotinus coggyria*) Cultivation

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ABSTRACT

This research aims to develop a novel nano-fertilizer additive through the use of nano-technological principles. The synthesis of iron nanoparticles was carried out using water-soluble iron salt and pomegranate plant extract as a green synthesis reaction medium, thus creating an innovative and environmentally friendly approach. A comprehensive analysis of the synthesized particles was carried out using various advanced techniques such as SEM, TEM, XRD, FT-IR, and UV-Vis, elucidating their complex morphological and elemental properties and confirming their nanoscale size. Then, the application of the produced nano-fertilizer on specific plant species (Smoke and Flame Tree) was investigated to evaluate its effect on plant growth. In this context, the nano-fertilizer was applied to plant tissue cultures grown under controlled and sterile conditions in the plant tissue laboratory, followed by a comparative analysis with control groups. The results of the study revealed the superior efficacy of nano-iron based nano-fertilizer, which requires significantly less amount of iron compared to conventional iron fertilizers. Multiple parameters were considered for the analysis of the results. In particular, the application of nano fertilizer gave more favorable results for the growth of the Flame tree (*Photinia fraserii*) when the nanodimer was used at a ratio of 1/46 to the standard iron-containing medium. Similarly, for the Smoke tree (*Cotinus coggygria*), favorable effects on plant growth were observed at a ratio of 1/34 compared to the standard iron-containing medium. These findings suggest that nano-fertilizer formulations containing synthesized nano-iron additives can increase agricultural yield, minimize environmental impact, and contribute positively to overall plant production.

INTRODUCTION

Crop production is a very important area of research today. The world's population, faced daily with scarcity and environmental problems, has begun to look at the rational management of agricultural land. For this reason, a large number of studies are carried out to obtain high yields from arable lands. Most of these studies are carried out abroad. For this reason, it is very important to examine and develop the agricultural applications of nanotechnology, which has a high material value for our country and is an area that will reduce our foreign dependence (Ebrahiminezhad et al., 2017; Sekhon, 2014). To this end, minimizing soil pollution caused by excessive fertilization while minimizing environmental damage is a very important research topic. The objectives of our study are to synthesize iron nanoparticles using the green synthesis method and to prevent both soil and water pollution by growing crops with a smaller amount of nano-fertilizer using this nanoparticle.

Nano-iron fertilizers produced through green synthesis have emerged as a potential alternative to traditional fertilizers in agriculture. These nano-fertilizers have been shown to enhance nutrient uptake and utilization in various crops. In the case of flame tree (*Photinia fraseri*) and smoke tree (*Cotinus coggygria*) cultivation, the use of nano-iron fertilizers could potentially improve growth performance and nutrient absorption. The green synthesis method used to produce nano-iron fertilizers is advantageous as it avoids the use of expensive and potentially harmful chemical methods. Green synthesis means that chemical processes are carried out by living organisms or parts of organisms (e.g. extracts, biological molecules). Thus, it is possible to synthesize nanoparticles easily and with high efficiency. Nanobiotechnology, which emerged with the use of living structures in nanotechnology, is an important research field. In this field, nanoparticles can be synthesized by using living organisms or their parts, such as plant extract, bacteria, fungi, yeast, algae, viruses, etc., as reaction media for synthesis (Ebrahiminezhad et al., 2017; Feng et al., 1999; Sekhon, 2014). Nano-fertilizer can be defined as a nano-material consisting of one or more components that promote plant growth and development (Liu & Lal, 2016), Nano-fertilizers are more advantageous than other fertilizers in many ways. Due to their size, nano-fertilizers can be more easily and more easily taken into the plant from the surface where they are applied. This increases the use of fertilizer by the plant. Different types of nano-fertilizers have been developed and used until today. Some of them can be in the form of nanoparticles, nanoemulsions, nanocapsules or nanofilms, or nanotubes (Jampilek & Kralova, 2015; Singh et al., 2017; Valizadeh & Milic, 2016). Nano-fertilizers provide plants with the building blocks necessary for healthy growth and development. In this way, the plant develops more resistance to the negative conditions of the external environment and gains resistance.

Fertilizers used in agriculture can be applied to the plant by different methods. These methods are mostly coding to the seed, adding to the soil as well as spraying the leaves of the plant. In this way, nano-fertilizers that are used less or sprayed on the leaves will be used in a way that will cause less damage to the environment (Mukherjee et al., 2015). When the seed coding process is performed using nano-fertilizer, difficult germinating seeds will be germinated very easily, thus easier growth and yield will be obtained.

In addition, the resistance of plants against pathogens increases if these elements are sufficiently taken by plants. Since the pH of the soils is neutral and alkaline, the entry of minerals into the plant is less in alkaline environments. However, nanoscale minerals can be easily taken into the plant by passing through the stomatal spaces of the plant with almost 100% efficiency. Some of the metals used as nano-fertilizers are (Zn, Cu, and Fe). Some publications in the literature emphasize the necessity of developing nano-fertilizers for the above reasons (Daghan, 2017; Rameshaiah et al., 2015). Rui et al. used fertilizers containing nanoiron instead of iron in peanut production and investigated their effects on plant production. As a result of their investigations, it was reported that iron nanoparticles were much more effective than iron in many aspects (Rui et al., 2016).

Another research group examined the effect of nano Fe, K, and P on saffron plants against the control group. From their findings, they concluded that nano fertilizers are very important for the production of a plant such as saffron, which is quickly affected by environmental factors (Amirnia et al., 2014). In a study conducted on the Propeller flower, an ornamental plant; the effects on plant growth were discussed comparatively by using iron and nano iron-containing fertilizers (Askary et al., 2016).

Plant-mediated synthesis of iron nanoparticles has been explored as a sustainable and eco-friendly approach. This method involves the use of plant extracts to reduce and stabilize iron nanoparticles, making it a promising technique for the production of nano-iron fertilizers. Some of the most important factors in the preference for the green synthesis method are the low use of toxic substances and the ability of the method to perform synthesis under mild conditions. In addition, due to their low toxic substance content, they can be used more easily as bio-safes in many application areas, including health and medicine. For a reaction to be a green synthesis, it must be safe, one-step, produce minimum waste, be environmentally friendly, the product can be separated from the reaction medium in a simple way, be highly efficient, and have some of the features such as the use of renewable materials in the reaction medium. Metal nanoparticles can be synthesized in different reaction media using the green synthesis method. Reaction media such as herb (*Stevia rebaudiana*) are used as reducing agents, and metal nanoparticles are synthesized (Bar et al., 2009; Ekinci et al., 2014; Filippo et al., 2015; Mishra et al., 2010). Furthermore, the application of mineral foliar treatments has been shown to have positive effects on plant responses to various stressors, such as salinity. Integrated foliar applications of potassium (K) and zinc (Zn) have been found to mitigate the detrimental effects of salinity on plant growth and yield (Chrysargyris et al., 2018). This suggests that the foliar application of nano-iron fertilizers, in combination with other mineral nutrients, could potentially enhance the growth and productivity of flame trees and smoke trees under challenging environmental conditions.

The use of nano-iron fertilizers produced through green synthesis holds promise for improving the cultivation of flame trees and smoke trees. These fertilizers have the potential to enhance nutrient uptake and utilization, leading to improved growth performance. In addition, the plant-mediated synthesis of iron nanoparticles offers a sustainable and environmentally friendly approach to the production of nano-iron fertilizers. Further research is needed to determine the optimal application methods and concentrations of nano-iron fertilizers for these specific plant species. In addition, metal nanoparticles were synthesized by our group using different plant extracts. These synthesized nanoparticles have found use in many different fields with their morphology and characteristics (Nalcioglu et al., 2019; Osman et al., 2024; Singh et al., 2018; Wang & Nguyen, 2018).

Our country is one of the most important pomegranate producers. Pomegranate production is increasing in direct proportion to its usage areas. Consumption demand, which is a factor in increasing production, includes factors such as different food technologies, transport, storage, and packaging (Karaaslan et al., 2014). In addition to modern methods, traditional methods are still used to produce pomegranate extract. This method is based on concentrating pomegranate juice by boiling it without adding any additional additives. Briefly, the production method is based on pomegranate fruit extraction, cleaning, crushing, filtration, and concentration by boiling (Francesca et al., 2014; Saeed et al., 2014; Xichuan et al., 2018). Pomegranate sour is an herbal product obtained by using the pomegranate plant and has both traditional and commercial applications. It has been determined that the ratio of phenolic components in the structure of pomegranate extract is quite high. In addition, it has been observed that many carboxylic acids are present in the structure (Liu et al., 2021; Saparbekova et al., 2023). In this research planned by our group, pomegranate fruit extract was used for nano iron production as a reductant in green synthesis.

The vegetative production method is widely used in ornamental plant production. Using plant tissue culture instead of traditional production is therefore very important (Demirbas et al., 2020). Plant tissue culture is the process, under sterile and controlled conditions in an artificial culture medium, of producing a new plant, tissue, or various secondary metabolites from a cell, tissue, or organ taken from the parent plant (Salam et al., 2023). This method is widely used. It allows for faster production compared to traditional cultivation techniques (Orcan & Akbas, 2020). In addition, endemic, economically valuable, genetically high-yielding individuals can also be produced quickly, disease-free, and virus-free Ornamental plants are plants used for gardening and landscaping. They are widely used for landscape ornamentation, especially in park garden areas. We planned to conduct our research on two important model plants selected from these;

Smoke tree; *Cotinus coggyria* belongs to the plant kingdom, branch *Angiospermae*, subclass Rosidae, order Sapindales, family Anacardiaceae. *C. coggyria* is a deciduous shrub up to 5 meters tall. The leaf structure is simple and the flowers are hermaphrodite. The smoke tree, which is naturally distributed in maquis and bushes, is grown in gardens as an ornamental plant (Rovina et al., 2013). Pharmacological and biochemical components are obtained from the essential oils and extracts found in the flowers, shoots, leaves, and stems of the plant. These compounds have antioxidant, anticancer, antibacterial, antiviral, antigenotoxic, antifungal, hepatoprotective, and anti-inflammatory properties. Therefore, in addition to its ornamental value, *C. coggygria* has important medicinal properties (Pacholczak et al., 2015; Silva et al., 2018).

Flame tree; *Photinia frasserii* belongs to the plant kingdom, Angiospermae section, Rosidae subclass, Rosales order, Rosaceae family. P. Frasserii is an evergreen woody ornamental plant that can grow up to 3-5 meters tall. It is widely preferred in seedling cultivation with its leaf shape, colors, and fast growth. It is one of the most remarkable ornamental plants thanks to its long, straight, and shiny, resistance to cold weather conditions and the most extreme leaves with intense red color (Jianhua et al., 2019). With its impressive leaves and resistance to adverse environmental conditions, the economic and environmental value of the species is increasing day by day (Akdemir, 2016). It has been shown that vegetative propagation by traditional methods is inefficient and rooting of this species is difficult. The available information on the application of known in vitro techniques for the reproduction of this species is very limited (Monica et al., 2021). Studies clearly show that the use of nano fertilizers has many properties in terms of crop yield, environmental protection, and plant resistance. Nano-components can be used by themselves or they can be mixed with micro-elements to create a synergistic effect. The most important handicap of nano-fertilizer applications is determining the dosage. For this reason, one of our aims in this study is to determine the application dosage of iron nanoparticles. One of the most important objectives of this research is to develop a nano fertilizer component produced in our country using domestic resources and to investigate its use in model plants.

MATERIALS AND METHODS

Preparation of Pomegranate (*Punica granatum*) Extract

Pomegranate (*Punica granatum*) was purchased from local markets in Erzurum province and kept in the refrigerator at +4^{o}C until the study. Afterwards, the pomegranate was crushed into pieces and the solid particles were separated from the solution by filtration. Approximately 2-2.5 liters of pomegranate juice was concentrated to a final volume of 100 mL using an evaporator. In this way, the formation of hydroxy methyl furfual (HMF) in the structure of pomegranate sour was prevented.

Green Synthesis of Fe_2O_3 Nanoparticles

10 mL of pomegranate extract was added to 1000 mL of 10 mM $FeCl_3$ solution and the solution was monitored in a spectrophotometer for 4 hours. The presence of iron nanoparticles formed with time in the solution was detected by dark red color. The synthesis method developed for iron nanoparticles was optimized and shown in Figure 1.

Reaction medium

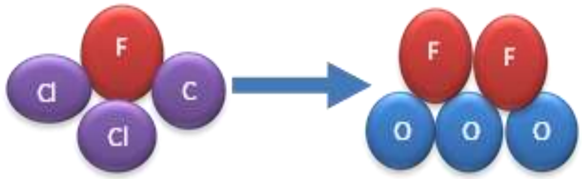

Figure 1. Fe_2O_3 green synthesis using pomegranate extract

Characterization of Nanoparticles

It was carried out at the Eastern Anatolia High Technology Application and Research Centre (DAYTAM) of Atatürk University. The equipment used for characterization is listed below: Transmission Electron Microscope (TEM): Hitachi 7700 model, Scanning Electron Microscope (SEM) for SEM analysis: Zeiss brand Sigma 300 model, X-ray diffraction (XRD): Panalytical brand Empyrean model and Fourier transform infrared spectrophotometer (FT-IR): Bruker model Vertex 70 was used. As a result of the analysis, information about the size and morphological properties of the synthesized nanoparticles was obtained.

Tissue Culture Stages

This research was conducted by the research group under the responsibility of Prof. Dr. Taki DEMIR at Sakarya University of Applied Sciences, Plant Tissue Culture Laboratory (Figure 2). Nano iron was applied in vitro on Smoke tree (*Cotinus coggygria*) and Flame tree (*Photinia frasserii*) plants in tissue culture.

Figure 2. Sakarya University of Applied Sciences, Plant Tissue Culture Laboratory

Sterilization of Equipment

The glass, ceramic, and metal materials used in the study were sterilized in an oven at 180 °C for 1 hour, and the media used in the inoculation process were sterilized by autoclaving at 121 °C for 20 minutes at 1 atm pressure.

Preparation of Media

MS (Seon et al., 2024) and DKW (Morales et al., 2024) media were prepared by adjusting the pH to 5.6-5.8 using high-purity chemicals. The media were divided into glass jars (60 mL) using a liquid filling machine and sterilized in an autoclave (Gungor et al., 2018).

Plant Treatments

MS and DKW media were used for the smoke tree (*Cotinus coggygria*) and Flame Tree (*Photinia frasserii*), respectively. The plants were sown in different iron-containing media in a sterile cabinet and cultured in a climate chamber at 24±2 °C for 30 days under a 16-hour light photoperiod. The experiment was carried out according to the randomized plots experimental design with 5 replications. To better discuss the results of the experiment, plant tissue cultures established from samples containing 2-10 mg/L nano iron (D1, D2, D3, D4, and D5), control without iron addition (K) and standard iron addition (D6) were analyzed.

As a result of the experiments, the plants obtained as a result of the media trials in the micropropagation studies of the plants were evaluated in terms of the number of shoots per explant (number), shoot length per explant (mm), plant stem length (mm), internode (mm) and number of leaves (number). All plant trials were subjected to Duncan's multiple comparisons and analysis of variance in the SPSS statistical software.

RESULTS AND DISCUSSION

It has been seen from our previous literature research that phenolic compounds and carboxylic acids in the structure of pomegranate plants are used in nanoparticle formation (Al-Timmimi, 2017). For this reason, it was aimed to synthesize iron nanoparticles using the green synthesis method with pomegranate extract by taking advantage of the properties of its structure. Then, it was investigated whether the synthesized nano iron particles could be used as fertilizer additives.

Green Synthesis of Fe_2O_3 Nanoparticles

Iron oxide nanoparticles obtained under optimal conditions using pomegranate extract were washed and dried and then characterized.

Characterization of Nanoparticles

The SEM and FT-IR results obtained are given below in Figures 3a and b respectively.

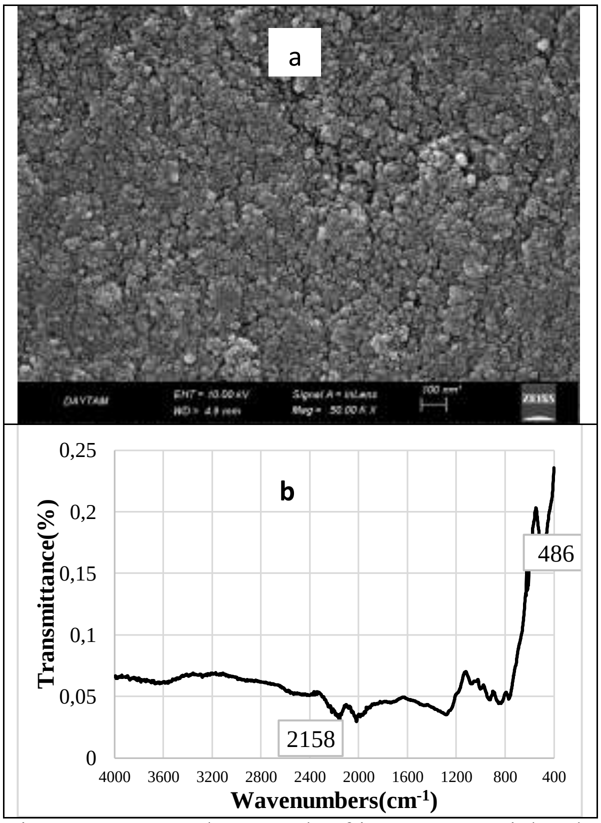

Figure 3a. SEM photograph of iron nanoparticles, b FT-IR spectrum of iron nanoparticles

The characterization of Fe_2O_3 nanoparticles obtained by the green synthesis method was carried out using SEM and FT-IR analysis and the results are shown in Figure 3. In SEM images, it is clearly seen that iron oxide nanoparticles are nanosized (Rizwan et al., 2017). As a result of the analysis, it is understood that Fe_2O_3 nanoparticles have a spherical structure with dimensions of 20-30 nm. In the FT-IR spectrum, the characteristic peaks of Fe_2O_3 nanoparticles are seen around 486 nm.

When the previous studies were examined, it was seen that silver nanoparticles were synthesized using pomegranate juice in the research conducted by the group (Mona et al., 2016). In another study, it was reported that gold nanoparticles were synthesized using pomegranate (Basavegowda et al., 2013). In another study, Fe_2O_3 nanoparticles were obtained using pomegranate seed extracts. For this, the organic components in the oils contained in the seed extract were found to be effective (Bibi et al., 2019). In the green synthesis with pomegranate extracts, it was understood that phenolic compounds and carboxylic acids in the structure were involved in the reaction (Al-Timmimi, 2017).

Plant Treatment

DKW medium was used for the flame tree. These plants, planted in a sterile cabinet in different iron-containing media, were grown in a climatic chamber at 24±2°C for 30 days under a 16-hour light photoperiod. The experiment was set up according to the randomized design with 5 replications.

Table 1. Plant growth parameters in the flame tree (*photinia frasserii*) nano iron trials

Experiment groups	Number of Shoots per Explant (explant/shoot)	Plant length (mm)	Shoot length (mm)	Internode length (mm)	Leaf number (leaf/explant)
Control	2,53 [c]	16,54 [b]	4,67 [a]	3,40 [a]	15,40 [c]
D1	9,00 [ab]	14,10 [b]	4,17 [ab]	2,93 [ab]	31,46 [b]
D2	8,40 [b]	13,55 [b]	3,82 [bc]	3,10 [ab]	30,86 [b]
D3	10,13 [a]	15,14 [b]	3,78 [bc]	2,85 [ab]	41,66 [a]
D4	8,13 [b]	21,92 [a]	4,13 [abc]	3,40 [a]	30,40 [b]
D5	8,06 [b]	12,53 [b]	3,37 [c]	2,58 [b]	29,80 [b]
D6	8,93 [ab]	15,80 [b]	3,89 [bc]	2,66 [b]	35,86 [ab]

a-e: The comparison between the samples in the same column is statistically meaningful ($P<0.05$).

In Table 1, the largest number of shoots per explant was produced by D1, D3, and D6 (a maximum of 10.13 shoots per explant). It was found that there was no statistically significant difference between D1, D3, and D6 media in the number of shoots per explant. The highest plant length was determined in the D4 medium with 21.92 mm. Although the highest shoot length was in the control group with 4.67 mm, the plants had a very light green color and spindly stem structure. Iron deficiency causes a brittle condition called cholorosis in the plant (Li et al., 2021). There was no statistically significant difference between the control and D1 and D4 environments. Internode distance gave the same results with 3,40 mm in control and D4 media. However, abnormal development in the control group caused unhealthy plant formation. The highest number of leaves was 41.66 in the D3 medium. According to these results, D3 was found to be the best medium for plant growth and reproduction. The amount of Fe used in the standard medium is about 5 times the amount of nano iron used in the D3 medium.

MS growing medium was used for the smoke tree (*Cotinus coggygria*). The plants were sown in different iron-containing media in a sterile cabinet and cultivated in a climate chamber at 24±2 0C with 16 hours of light photoperiod for 30 days. The experiment was established according to the randomized plots experimental design with 5 replicates.

Table 2. Plant growth parameters in the smoke tree (*Cotinus coggygria*) nano iron trials

Experiment groups	Number of Shoots per Explant (explant/shoot)	Plant length (mm)	Shoot length (mm)	Internode length (mm)	Leaf number (leaf/explant)
Control	0,33 [e]	13,64	2,27 [ab]	2,75	6,66 [d]
D1	0,40 [e]	19,53	1,18 [b]	3,16	7,33 [cd]
D2	1,46 [cd]	14,55	2,71 [ab]	3,53	9,00 [cd]
D3	2,50 [b]	15,48	2,65 [ab]	2,81	13,51 [abc]
D4	1,80 [bc]	17,04	3,14 [ab]	2,52	18,33 [a]
D5	3,46 [a]	15,31	3,85 [a]	2,90	15,46 [ab]
D6	0,80 [de]	15,02	3,19 [ab]	3,08	9,60 [bcd]

a-e: The difference between the samples in the same column is statistically significant ($P<0.05$).

In Table 2, the largest number of shoots per explants was achieved by the D5 medium with 3.46 mm of shoots per explants. It was observed that there was no statistically significant variation in the number of shoots per explant between the control, D1, and D6 media. There was no statistically significant difference between the media in terms of plant height and internode length. The highest shoot length was 3.85 mm in D5 medium. When shoot length was taken into consideration, it was determined that there was no statistically significant difference between the other environments except the D1 environment. The highest number of leaves was 18.33 in the D4 medium. There was no statistically significant difference between D3, D4, and D5 environments in terms of the number of leaves. According to these results, D5 was found to be the best medium for plant growth and reproduction. In the D4 medium, nanodimer was used at approximately 1/34 of the amount of Fe in the standard medium.

Likewise, Tung et al. (2020) have investigated the impact of Fe_2O_3 NPs in place of Fe-EDTA on the growth and antioxidant activity of MS medium. They showed that Fe_2O_3 NPs added to the MS medium had higher growth and antioxidant activity than Fe-EDTA in the MS culture medium. In this study, low nano-iron was found to increase the number of shoots per explant in the plant(Tung, 2020).

Kokina et al. in their study with yellow medick plants, grew the plants for five weeks in a hydroponic medium containing Fe_2O_3 NPs. They found that Fe_2O_3 NPs decreased the root length of plants (9-32 %), chlorophyll, and fluorescence (1.94-2.80 fold) and induced genotoxicity and genome stability (12.50 %) compared to the control.

Their research found that the in vitro use of Fe2O3 NPs also had positive effects on plant growth (Kokina, 2020; Twardovska et al., 2024). Iranbakhsh et al. (2020) found that Fe_2O_3 NPs increased biomass accumulation in the roots and shoots of pepper seedlings. Fe_2O_3 NPs also increased photosynthetic pigment concentrations (Iranbakhsh, 2020).

Low doses of Fe_2O_3 NPs used in vitro increase plant tolerance to other stress factors, especially salinity, in addition to its positive effect on plant growth In this case, the use of low dosage of fertilizers is the most appropriate and sustainable solution to prevent the pollution of the natural environment.

In this way, environmentally friendly and sustainable plant nutrition can be provided (Mozafari, 2018), in their study with strawberry plants, determined that the application of Fe_2O_3 NPs improved all growth-related parameters (Mozafari, 2018). They found that the levels of pigment, iron, and potassium in the mature plants treated with the nanodimer used increased and that the sodium content decreased under conditions of high salinity. Asl showed in his study that moderate concentrations of iron nanoparticles can be used to increase salinity tolerance (Asl, 2019).

CONCLUSION

From the experimental data obtained by these methods, the opportunity was obtained to examine and reveal the effects of iron nanoparticles prepared using pomegranate extract by green synthesis method on plant growth. In line with the data obtained, it was seen that the newly synthesized nano-fertilizer additive will have positive effects on model plants and different plants and increase productivity. In addition, in line with the results obtained, it is thought that it will be a study that guides and forms the basis for the use of nano-fertilizers in plant growth and propagation, especially ornamental plants. It was understood that nano-iron obtained by green synthesis can be used as fertilizer formulation.

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