International Journal of Innovative Research and Reviews, 6(1) 6-10

International Journal of Innovative Research and Reviews
ISSN: 2636-8919
Website: www.injirr.com
doi:
Research paper, Short communication, Review, Technical paper

RESEARCH ARTICLE

Synthesis of Nano Iron Oxide and Investigation of Its Use as a Fertilizer Ingredient

Neslihan BABALI[1,*] Azize ALAYLI[2] Hayrunnisa NADAROĞLU[3,4] Taki DEMİR[1]

[1] Department of Horticulture, Faculty of Agriculture, Sakarya University of Applied Sciences, 54580 Sakarya, Turkiye
[2] Department of Nursing, Faculty of Health Sciences, Sakarya University of Applied Sciences, 54187 Sakarya, Turkiye
[3] Department of Food Technology, Vocational College of Technical Sciences, Ataturk University, 25240 Erzurum, Turkiye
[4] Department of Nano-Science and Nano-Engineering, Institute of Science, Ataturk University, 25240, Erzurum, Turkiye

** Corresponding author E-mail: neslihanb@subu.edu.tr*

HIGHLIGHTS

> Iron nanoparticles were synthesized by green synthesis method and morphology analyzes were performed by SEM.
> The use of obtained iron nanoparticles as nano fertilizer was investigated in tissue culture medium.
> It was found that nano iron can have positive effects on the growth of Chinese fringe flower (Loropetalum chinense).

ARTICLE INFO

Received : 12.11.2021
Accepted : 04.18.2022
Published : 07.15.2022

Keywords:
Green synthesis
Iron oxide
Micro-propagation
Nano-particles

ABSTRACT

Faced with famine and environmental problems day by day, the world population has begun to explore the rational use of arable land. It is very important to minimize soil pollution caused by excessive fertilization used in this area. In this study, pomegranate fruit extract was used as the reducing medium of the green synthesis method. After characterizing the nano-ironoxide particles (Fe_2O_3 NPs) produced under optimized conditions, its effects on plants were investigated. To examine the effects of Fe_2O_3 NPs on plant growth, experimental random plots were set up with the plant Chinese Fringe Flower plant (*Loropetalum chinense*) grown under sterile and homogeneous conditions. The number of shoots per explant, which was 2.76 in the standard iron-containing medium, was obtained as 4.00 in the Fe_2O_3 NPs-1.00 mg/L medium. These results, it was determined that the best medium for plant growth and reproduction was Fe_2O_3 NPs-1.00 mg/L. For this reason, it was thought that domestic nano fertilizer formulations can be used to increase agricultural productivity and create value-added contributions to countries in the production of different plants that cause the least damage to the environment.

Contents

Cite this article Babalı N, Alaylı A, Nadaroğlu H, Demir T. Synthesis of Nano Iron Oxide and Investigation of Its Use as a Fertilizer Ingredient. *International Journal of Innovative Research and Reviews (INJIRR)* (2022) 6(1) 6-10
Link to this article: http://www.injirr.com/article/view/90

1. Introduction

Crop farming is a very important research field today. Today, 78% of the plants produced are ornamental plants [1]. The world population, which is facing famine and environmental problems day by day, has started to search for rational methods of arable agricultural lands. It is very important to minimize soil pollution caused by excessive fertilization with minimal damage to the environment. Nutrient element (Fertilizer); it affects the plant growth pattern by changing the plant morphology, anatomy and especially its chemical composition, and accordingly, it can increase or decrease the resistance and tolerance of plants against diseases and pests. Plant nutrients are chemical elements and are essential for plant growth [2]. The use of nano-fertilizer has many features in terms of crop yield, environmental protection and plant resistance.

There are nanomaterials in everything from the tools we use to our clothes to personal care products. Today, nanotechnology has taken its place in many branches of science, such as biology and chemistry, and in almost every area of our lives. In tissue culture, studies have been carried out with very successful results using nanotechnology products. Nanoparticles are used to provide seed germination, increase plant growth and yield, increase the amount of plant secondary metabolites and provide plant protection from various factors [3].

Chinese fringe flower plant (*Loropetalum chinense*), belongs to family of *Hamamelidaceae* [4]. Its natural distribution area especially with Hunan province being the center in China [4–8]. The different pieces of the plant are known to be used in traditional Chinese medicine for the treatment of bleeding disorders, burn treatment, skin infections, various gynecological diseases, dysentery and diarrhea. It is known to have hemostatic, antipyretic and detoxifying properties. Through to agency of tannins it contains, it has antioxidant and bacteriostatic properties [4, 6, 9]. The commonly used method in ornamental plant production is the vegetative method. Therefore, it is very important to use the plant tissue culture method instead of traditional production [10]. Plant tissue culture; It is defined as the production of a new plant, tissue or various secondary metabolites from a cell, tissue or organ taken from the main plant under sterile and controlled conditions, in an artificial nutrient medium [11].

This method is widely used because it provides faster production compared to traditional cultivation techniques [12]. In addition, endemic, economically valuable, genetically and highly productive individuals are reproduced. Fast, disease-free and virus-free plants can be produced [13]. Chinese fringe flower is a very difficult plant to be grown by seed or vegetative methods. Chinese fringe flower is one of the most imported ornamental plants, which has been widely cultivated in landscaping in Turkey for the last seven years. However, the difficulties in the production of this plant, which is difficult to produce, can be overcome with tissue culture [14].

In order to examine the effects of Fe_2O_3 NPs on plant growth, experimental randomized plots were set up with Chinese fringe flower plant grown under sterile and homogeneous conditions. It is thought that domestic nano-fertilizer formulation additives with added value for our country can be created and used in the production of different plants, with minimum damage to the environment, to increase agricultural productivity. The aim of this study is to reduce the amount of iron used in in vitro plant growth, to see its positive effects on plant growth and to contribute to the national economy with less chemical use. For this purpose, the effect of the number of shoots per explant in different rates Fe_2O_3 NPs and the average growth performance of growing shoots on plant reproduction were investigated.

2. Experimental

2.1. Supply of chemicals and sterilization of equipment

All chemicals used during the experiment were obtained and used in analytical purity. Deionized water was used to prepare the solutions and media to be used. Glass, ceramic and metal materials used in the research were sterilized in an oven at 180 ºC for 1 hour, and the nutrient media to be used in the sowing process were sterilized by using autoclave at 121 ºC at 1 atm. pressure for 20 minutes.

2.2. Preparation of green synthesis medium with pomegranate (*Punica granatum*) plant

Pomegranate fruit (*Punica granatum*) was obtained from the local markets of Erzurum province in the August-September 2020 season and was kept in the refrigerator at +4 ºC until it was used. The pomegranate fruit was washed using distilled water so that the dust and dirt on it were removed. 5 liters of pomegranate juice obtained by squeezing is concentrated to a final volume of 100 mL and stored in the refrigerator at +4ºC until the obtained concentrated pomegranate is used.

2.3. Green synthesis of Fe_2O_3 nanoparticles

10mL of pomegranate concentrate obtained from pomegranate fruit (*Punica granatum*) will be added to 1000mL of 10 mM $FeCl_3$ solution. The mixture containing $FeCl_3$ solution and plant extract will be monitored in the spectrophotometer for 4 hours. During this time, the reaction mixture was stirred at 600 rpm at room temperature using a magnetic stirrer. The solution will be recognized when a deep red color is seen, indicating the presence of iron nanoparticles at the end of the specified time. The synthesis method developed for iron nanoparticles will be optimized and used. For optimization, parameters such as pH, temperature and concentration will be examined at 580 nm using UV spectrophotometer, and synthesis will be carried out under optimal conditions where synthesis is the best [15–18].

2.4. Characterization of nanoparticles

It was held at the Eastern Anatolia High Technology Application and Research Center (DAYTAM) within Atatürk University. Scanning Electron Microscope (SEM) was used for characterization. In this way, information about the size and morphological properties of the synthesized nano-particles were obtained [16, 19, 20].

2.5. Tissue culture application

In this research, Sakarya University of Applied Sciences, Faculty of Agriculture, Plant Tissue Culture Research and Production Laboratory. In vitro Chinese fringe flower plant (*Loropetalum chinense*) was used.

2.6. Media preparation

In order to prepare the medium, the medium was prepared as in Table 1. using high purity chemicals. All chemicals were weighed in the amounts specified on a precision scale and dissolved in pure water with the help of a magnetic stirrer. The obtained medium was given to the Chinese fringe flower plant at different concentrations by adding it to the medium in tissue culture medium and the effects on the growth of the plant were determined by measuring different parameters.

3. Results and Conclusions

The SEM image of the iron nanoparticles synthesized using the pomegranate plant was taken and shown in Figure 1. As seen in the SEM image, nanoparticles have a spherical morphology and have dimensions between 20-35 nm.

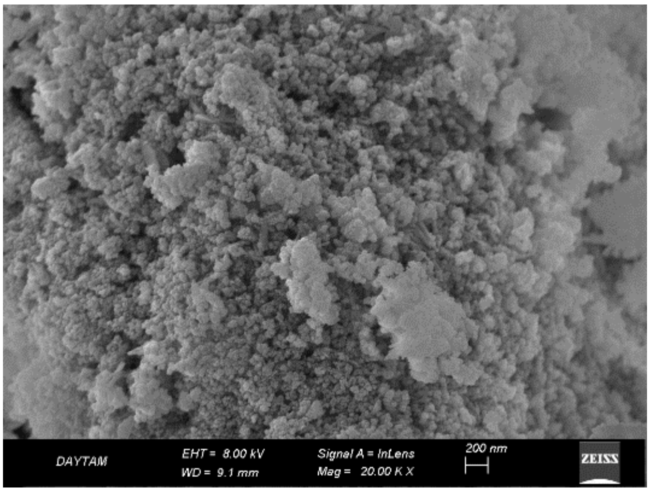

Figure 1 SEM image of Fe_2O_3 NPs

In this study, Chinese fringe flower plant in Sakarya University of Applied Science Plant Tissue Culture Laboratory was used. The nutrient media MS-modified used in the study [21]. The nutrients were sterilized in autoclave at 121°C for 20 minutes (1 atm). Explants taken from the mother plant were first washed under running tap water for 10 minutes and pre-sterilized with antifungal (Benomyl) for 15 minutes. It was then washed with 15% by volume sodium hypochlorite (ACE) for 15 minutes. Then, after washing with sterile distilled water. Culture medium explants were inoculated onto media composed of basal MS-mod [18] (Table 1) medium supplemented with the plant growth regulators;(6-benzylaminopurine) BA 1.00 mg/l and (Indole-3-butyric Acid) IBA 0.01 mg/l. Sucrose (3%) was used as carbon source and media were solidified with agar-agar (0.70%). The pH was adjusted to pH 5.70 prior to autoclaving at 121°C for 20 min. 10 shoots per culture bottle were inoculated and 5 replicates taken. The bottles were then kept in a growth chamber under 16:8 h (light: dark) photoperiod with light intensity of 2000-2500 lux provided 40 W white bulbs at 24±1°C.

Table 1 Nutrient media and ingredients (mg/L).

Ingredients	MS-modified (Babalı, 2020)
NH_4NO_3	500
KNO_3	2000
$Ca(NO_3)_2.4H_2O$	1200
$MgSO_4$	370
KH_2PO_4	170
$FeSO_4.7H_2O$	33.80
Na_2EDTA	45.40
$Na_2(MoO_4)$	0.39
$CuSO_4.5H_2O$	0.25
H_3BO_3	4.8
$Zn(NO_3)_2.7H_2O$	17
$MnSO_4.2H_2O$	33.5
$NiSO_4.6H_2O$	5
Glycine	2
Nicotinic acid	1
Thiamine HCl	2
My-inositol	1

Data were recorded after 4 weeks of culture. To examine the effects of Fe_2O_3 NPs on plant growth, experimental random plots were set up with the plant Chinese Fringe Flower plant grown under sterile and homogeneous conditions. All plant trials were subjected to variance analysis and Duncan multiple comparison in SPSS statistical program (at $p<0.01$).

Table 2 Chinese fringe flower plant development.

Plant media	Shoots per explant	Plant length (mm)	Shoot length (mm)	Internode (mm)	Leaves per explant
Fe_2O_3 NPs -0.00 mg/L	0.24^{e}	12.08^{b}	0.40^{d}	3.04^{b}	2.88^{d}
Fe_2O_3 NPs -0.20 mg/L	1.32^{d}	14.24^{b}	2.10^{c}	3.68^{ab}	6.28^{c}
Fe_2O_3 NPs -0.40 mg/L	4.28^{a}	15.83^{ab}	4.46^{ab}	3.51^{ab}	17.96^{a}
Fe_2O_3 NPs -0.60 mg/L	3.04^{bc}	16.28^{ab}	3.76^{ab}	3.17^{ab}	12.64^{b}
Fe_2O_3 NPs - 0.80 mg/L	3.40^{abc}	12.21^{b}	2.85^{bc}	2.76^{b}	15.04^{ab}
Fe_2O_3 NPs - 1.00 mg/L	4.00^{ab}	19.24^{a}	5.02^{a}	4.04^{a}	17.48^{a}
Standard Iron- 33.80 mg/L	2.76^{c}	13.23^{b}	3.43^{abc}	2.94^{b}	13.32^{b}

a-e: The difference between the samples in the same column is statistically significant ($p<0.01$).

In Table 2, the highest number of shoots per explant was obtained from Fe_2O_3 NPs -0.40 mg/L medium with 4.28. It was determined that there was no significant difference between Fe_2O_3 NPs -0.40 mg/L, Fe_2O_3 NPs -0.80 mg/L and Fe_2O_3 NPs -1.00 mg/L media in terms of the number of shoots per explant. The highest plant length per explant was 19.24 mm from Fe_2O_3 NPs -1.00 mg/L medium. It was determined that there was no significant difference between Fe_2O_3 NPs -0.40 mg/L, Fe_2O_3 NPs -0.60 mg/L and Fe_2O_3 NPs -1.00 mg/L media in terms of plant length. The highest shoot length per explant was 5.02 mm from Fe_2O_3 NPs -1.00 mg/L medium. It was determined that there was no significant difference between Fe_2O_3 NPs -0.40 mg/L, Fe_2O_3 NPs -0.60 mg/L, Fe_2O_3 NPs-1.00 mg/L and Iron Standard-33.80 mg/L media in terms of shoot length. The highest leaf per explant was 17.96 and 17.48 from Fe_2O_3 NPs-0.40 mg/L and Fe_2O_3 NPs-1.00 mg/L medium. According to these results, it was determined that the best medium for plant growth and reproduction was Fe_2O_3 NPs-1.00 mg/L. It is a medium containing Fe_2O_3 NPs about 1/34 of the amount of Fe in the standard used medium.

Nhut et al. [22], investigated the effects of Fe_2O_3 NPs (Fe-nanoparticles) on growth and antioxidant activity instead of Fe-EDTA in the MS [23] medium. It shows that Fe_2O_3 NPs added to MS medium have higher growth and antioxidant activity than Fe-EDTA in MS medium. In this study, the number of shoots per explant in the low nano-iron plant increased. Kokina et al. [24], in their study with yellow medick plants, they were grown in hydroponic media containing Fe_2O_3 NPs for five weeks. Showed that Fe_2O_3 NPs reduced plant root length (9%-32%), chlorophyll a fluorescence (1.94-2.80-fold), induced genotoxicity, and genome stability (12.50%) compared to control. In this study, it was determined that the use of Fe_2O_3 NPs in vitro also had positive effects on plant growth.

Iranbakhsh et al. [25], In their study, they found that Fe_2O_3 NPs increased biomass accumulation in roots and shoots in pepper plant seedlings. Also, Fe_2O_3 NPs increased the photosynthetic pigment concentrations. Asl et al, the results showed that moderate iron nanoparticle concentrations can be used to increase salinity tolerance [26]. Mozafari et al. [27], in their study with strawberry plant, it was determined that the application of Fe_2O_3 NPs improved all growth-related parameters. They observed that the pigment, iron and potassium contents of mature plants applied to the used nano-iron increased and the sodium content decreased under salinity conditions. Low amount of nano-iron used in our study may have a positive effect on plant growth as well as its tolerance to stress.

In addition to its positive effect on plant growth, low doses of Fe_2O_3 NPs used in vitro increase its tolerance to other stress factors, especially salinity. In addition, the use of low-dose fertilizer is the most suitable and sustainable solution to prevent environmental pollution. In this study, the positive effects of low use of nano-iron produced with environmental technology compared to the standard on plant growth were determined. In this way, environmentally friendly, sustainable plant nutrition can be provided. It is possible to produce and grow the Chinese Fringe Flower plant, which is highly preferred especially in the landscape, with less fertilization.

Acknowledgment

This research was carried out with the support of Sakarya University of Applied Sciences scientific research project (SUBU-BAP: 2021-06-03-044).

Declaration of Conflict of Interest

Authors declare that they have no conflict of interest with any person, institution, or company.

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