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Ferrite (Fe_3O_4) Nanoparticle in Soil Stimulates the Plant Growth in Peas and Bok Choy

Hạt nano ferit (Fe_3O_4) trong đất kích thích sự phát triển của thực vật ở cây đậu Hà Lan và cải ngọt

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Abstract

Iron oxide nanoparticles have been known to be non-toxic and are among the most widely used nanomaterials in life, from the medical, agricultural to environmental fields. However, so far, the understanding of the interaction of nanoparticles, in general, and iron oxide nanoparticles, in particular, with the environment and the flora and fauna ecosystems is still limited. This study evaluated the effects of ferrite (Fe_3O_4) nanoparticles in soil on the growth of peas (Pisum sativum) and bok choy (Brassica rapa). The study showed that the nanoparticle concentration of 25 mg/kg of soil had the best positive effect on peas growth in terms of the main root elongation and root water retention. At a concentration of 25 mg/kg of soil, iron oxide nanoparticles did not affect the dry biomass growth of root and plant in peas and bok choy, respectively, even in the presence of potassium sulfate in soil. This suggests that the effect of ferric oxide nanoparticles could be more dominant than that of potassium sulfate fertilizer while maintaining constant biomass with increasing water uptake. Further studies at the cellular and tissue levels are needed to better understand this issue.

Keywords: Iron oxide nanoparticle, ferrite, peas, bok choy, soil, plant growth.

Tóm tắt

Các hạt nano sắt ôxít đã được biết đến là không gây độc và là một trong những loại vật liệu nano được sử dụng rộng rãi nhất trong đời sống, từ trong lĩnh vực y tế, nông nghiệp cho đến môi trường. Tuy nhiên, cho đến nay, những hiểu biết về sự tương tác của các hạt nano nói chung và các hạt nano sắt ôxít nói riêng với môi trường và các hệ sinh thái động thực vật vẫn còn hạn chế. Nghiên cứu này đã đánh giá ảnh hưởng của các hạt nano ferit (Fe_3O_4) trong đất lên sự phát triển của cây đậu Hà Lan (Pisum sativum) và cây cải ngọt (Brassica rapa). Nghiên cứu chỉ ra rằng nồng độ hạt nano 25 mg/kg đất có tác động tích cực nhất đến sự phát triển của cây đậu Hà Lan về khả năng kéo dài rễ chính và giữ nước ở rễ. Ở nồng độ 25 mg/kg đất, các hạt nano sắt ôxít không ảnh hưởng đến sự phát triển sinh khối khô của rễ và cây tương ứng ở đậu Hà Lan và cải ngọt, ngay cả khi có sự có mặt của kali sunfat trong đất. Điều này cho thấy rằng tác động của các hạt nano sắt ôxít có thể chiếm ưu thế hơn so với tác động của phân bón kali sunfat với việc duy trì sinh khối không đổi trong khi tăng khả năng hút nước của rễ. Cần có những nghiên cứu sâu hơn ở cấp độ tế bào và mô để hiểu rõ hơn về vấn đề này.

Từ khóa: Hạt nano sắt ôxít, ferit, đậu Hà Lan, cải ngọt, đất, sự phát triển của thực vật.

1. Introduction

Iron oxide has long been considered non-toxic. In the past 20 years, this material has been found appearing in a wide range of applications. Iron, iron oxide, and magnesium nanoparticles are often advertised as being used for medical applications, such as dietary supplements. The medical field can be a traditional market for applications of iron oxide nanoparticles, where they can be found in a number of imaging techniques, gene therapy, drug delivery, and cancer treatment, especially in clinical diagnosis as diagnostic agents [1]. While traditional super-magnetic iron oxide nanoparticles can be found as diagnostic agents and new platforms in cancer treatment [2], non-electrostatic iron nanoparticles can be found mainly in applications aimed at improving the environment [3].

Along with the trend of applying nanotechnology in many different fields, the environmental industry also seeks to use nanotechnology to protect the environment as well as to provide effective solutions to clean the environment. The term nano-remediation has been born in recent years to refer to the application of nanotechnology in environmental remediation. For the purpose of transforming, detoxifying and/or decomposing pollutants, nano-processing methods often involve the application of nanomaterials such as nano zeolites, carbon nanotubes and nanofibers, bimetallic or metal oxide nanoparticles, etc. Among them, iron nanoparticles can be found mainly in applications aimed at improving the environment [3].

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A solution receiving great attention recently for the treatment of persistent organic pollutants is utilizing advanced oxidation processes employing iron oxide and H_2O_2 in the photo-Fenton reaction to promote the oxidation of those organic compounds. An approach using iron oxide nanoparticles combining with H_2O_2 producing plants to promote the formation of free OH radicals in the environment, thus enhancing the oxidation capacity towards persistent organic molecules has been proposed [4].

The rapid growth of applications with nanoparticles, of course, will come with new risks. Iron oxide nanoparticles using in any application will, in part, find their way either directly or indirectly end up in the environment. Key concerns include the possibility that nanoparticles can be released and accumulate in the environment, entering drinking water sources and foods inducing harm to human and animal health, their variability in the environment, and how they interact with and affect ecosystems.

Although concerns about the impact of iron oxide nanoparticles on human and animal health as well as ecosystems have been raised in the past few years, to date, still little information on the toxic effects of iron oxide nanoparticles has been published. Recent studies on different iron oxide nanoparticles have focused on their roles in the germination and budding pattern [5, 6], reproduction, regeneration, mass production [7], and root development [8,9] of some plants. Some studies have shed light on the biochemical and physiological activities of iron nanoparticles inside the plants [10].

It should note that the effects of iron and iron oxide nanoparticles on plant developmental patterns greatly depend on the characteristics (size, shape, surface charge, etc.) of the nanoparticles and how they are applied. More extensive studies on the effects of these materials in the environment on plants and ecosystems, thus, are needed in order to make reasonable adjustments and guidelines for solutions that use these iron nanoparticles in the environment. Concerning the appearance of these materials in soil, this study measures the effect of ferrite (Fe_3O_4) nanoparticles on the growth and root system development of peas (*Pisum sativum*) and bok choy *(Brassica rapa*) under in vivo conditions, as another step closer to the goal of understanding the behavior and role of iron oxide nanoparticles in soil. In addition, the effect of the nanoparticles on plant development under the presence of potassium sulfate (K_2SO_4), an effective enhancer for plant growth, has also been investigated.

2. Methods and Materials

2.1. Materials

The iron oxide nanoparticles used in this study were ferrite (Fe_3O_4), commercial magnetite nanoparticles (CAS 1317-61-9, Sigma-Aldrich, Germany). The soil was Tribat clean soil (Saigon Xanh Biotechnology Co. Ltd., Saigon, Vietnam), which was treated for pathogens and contains already minimum nutrients and necessary minerals for plant growth. The soil was purchased in bags of 10 kg from a local distributor. Peas seeds (*Pisum sativum*) and bok choy seeds (*Brassica rapa*) were purchased from a local provider (Rang Dong Co. Ltd., Hanoi, Vietnam). The seeds were clean and packed in closed tin bags.

2.2. Experimental Design

Experiments with peas were conducted in 500 ml disposable polypropylene cups (12.7 × 9 × 6 cm of height × mouth diameter × bottom diameter). The soil was naturally dried to the moisture content of less than 20% then weighed and mixed with iron oxide nanoparticles and/or potassium sulfate (K_2SO_4, CAS 7778-80-5, Xilong China) to different experimental concentrations before being put into polypropylene cups with equal weights. The concentrations of nanoparticles and potassium sulfate in each experiment are described below.

Experiment 1 (the effects of iron oxide nanoparticles at different concentrations on plant development): the soil was mixed with only the nanoparticles at 0, 25, 50, and 100 mg per kg of dried soil.

Experiment 2 (the combined effects of iron oxide nanoparticles and potassium sulfate on plant development): the soil was mixed with the nanoparticles and K_2SO_4 to the concentrations of 0/0, 25/0, 0/4.46, and 25/4.46 (Fe_3O_4 nanoparticle (mg)/K_2SO_4 (g)) per kg of dried soil.

Seeds of peas (*Pisum sativum*) were selected for equal in size before soaking in distilled water for 3 h. The seeds were then sowed in prepared cups with 3 seeds each with equal spacing. Each treatment was conducted in 21 cups. At each chosen time point, three cups were randomly picked up for measuring the developmental parameters of plants.

Experiments with bok choy (*Brassica rapa*) were conducted in pots of 24 × 24 × 20 cm (length × width × height). The soil was naturally dried to the moisture content of less than 20% then weighed and mixed with iron oxide nanoparticles at the concentration of 25 mg per kg of soil. Each pot contained 2 kg of soil with 12 - 15 seeds sown in rows with equal spacing. The seeds were pre-selected for equal in size and soaked in distilled water for 5 h before sowing. All experimental pots were placed outdoors in a wooden frame box of 1.8 × 1.2 × 1.4 m (length × width × height) with walls and roof covered by 0.5 mm-thick transparent nylon sheets. Each treatment was conducted in triplicate.

2.3. Experimental Conditions

The initial pH of the soil was 7 and the initial moisture was 28 %, measured with a soil tester (MS04, Sonkir, Hanoi, Vietnam). The ambient temperature observed ranged from 19 to 22 ºC at night and 25 to 29 ºC during the day; relative humidity was 80 - 90%. The soil moisture during experiments was controlled at around 70%. Illuminance was from natural sunlight at 1500 - 1600 lux average daytime recorded during the time of conducting the experiment.

2.4. Data Collection and Analysis

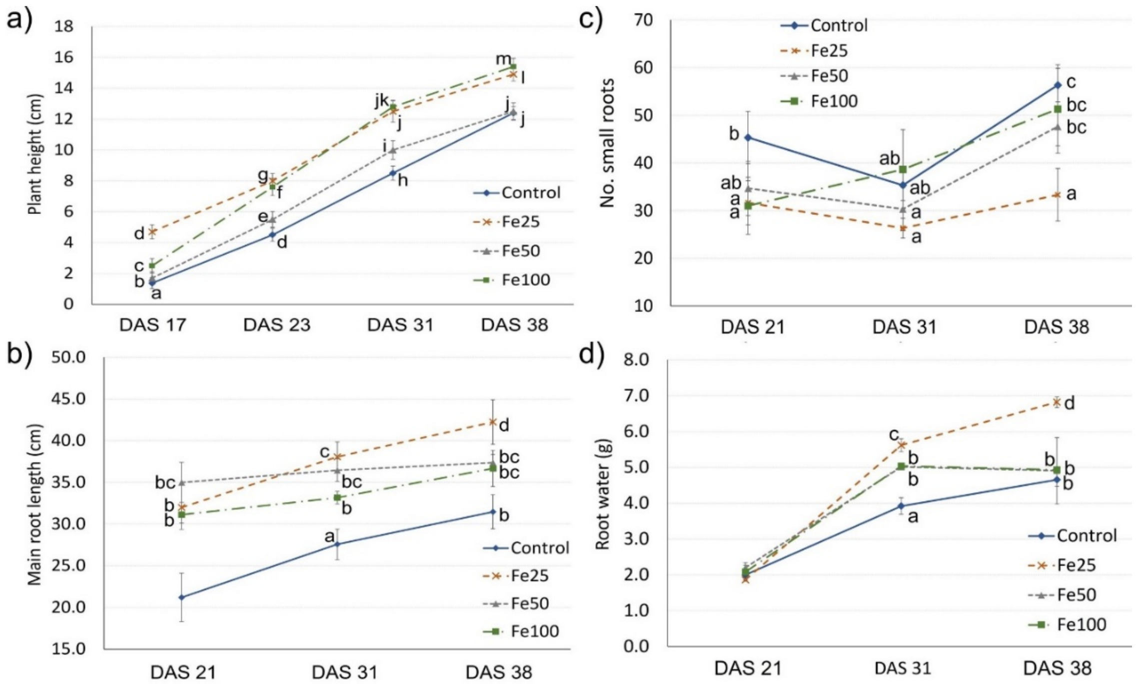
At certain time points (DAS, days after sowing) during the development of plants, some plants will be taken out to measure the set of growth parameters including plant height (cm, not including root), main root length (cm), small root number. For bok choy, data was collected at the day 38 after sowing. The data set includes the number of leaves.

To measure mass and water content of root (for peas plant) and the whole plant (for bok choy), the cut roots of the peas plant or the whole plants of bok choy were washed carefully with water to remove all soil particles, then dried naturally at ambient temperature. The roots and plants were then weighed for recording fresh mass. After that, they were continued to dry in a ventilated oven at a temperature of 40 - 45 ºC until the weight does not change. The final weights of roots and plants were recorded as their dry mass. Water content (m_{water}) was calculated as the difference of the fresh mass (m_{fresh}) minus the dry mass (m_{dry}) according to the following formula

$$m_{water} = m_{fresh} - m_{dry}$$

The number of seeds sown in each experiment was calculated so that at each sampling time, the number of plants that had been removed did not affect the number of plants remaining for subsequent samplings. A minimum of 5 plants was collected in each sampling session.

The data collected was imported into excel files. The statistical analysis of the data was verified with the one-way ANOVA. The Fisher LSD tests were used to compare the treatment's means. All analyses were conducted using the statistical package StatPlus LE Build 7.3.0.0 for Windows (StatPlus, AnalystSoft Inc., Walnut, CA 91789, USA). The significance level was set at 0.05.



Note	
Control	Original soil does not contain iron oxide nanoparticle
Fe25	Soil mixed with 25 mg iron oxide nanoparticle per kg of soil
Fe50	Soil mixed with 50 mg iron oxide nanoparticle per kg of soil
Fe100	Soil mixed with 100 mg iron oxide nanoparticle per kg of soil

Fig. 1. Effects of iron oxide nanoparticles at different concentrations in soil on the development of peas (*Pisum sativum*). DAS: days after sowing. Letter a, b, c, d, e, f, j, k, m, and l indicate significant differences (one-way ANOVA and Fisher LSD, $p \leq 0.05$).

3. Results and Discussion

3.1. Iron Oxide Nanoparticle Stimulating Peas (Pisum sativum) Development in Term of Root and Water Absorption Capacity

Data in Fig. 1 shows the effects of iron oxide (Fe_3O_4) nanoparticles at different concentrations in soil on the development of peas. It indicates that plants in the control case, where seeds were sown on original soil without nanoparticles, show lower rates of development compared to plants in other cases except for the parameter of the number of small roots. Plants in the case where seeds were sown on soil containing 25 mg iron oxide nanoparticles per kg of soil, interestingly, shows reversed trend where they show significantly higher rates in most developmental parameters including plant height, main root length, and the water content in the root (Fig. 1a, b, and d), except for the number of small roots (Fig. 1c). Plants in other cases where seeds were sown on soil containing 50 or 100 mg nanoparticles per kg of soil show no significant difference to the plants in the control treatment in all developmental parameters. Data reveals that plants in these two cases show increases in the main root lengths and water contents in roots during the first 31 days after sowing, but no significant difference to those of the plants in control case at 38 days after sowing. This suggests that under the presence of iron oxide nanoparticles at the concentration of 25 mg per kg of soil, the plants might prioritize the development of the main root that could help them have the ability to absorb more water from the soil. Based on this finding, the concentration of 25 mg iron oxide nanoparticles per kg of soil is chosen for further experiments.

3.2. Effects of Iron Oxide Nanoparticles on Peas (Pisum Sativum) Development under the Presence of Potassium Sulfate in Soil

Potassium sulfate (K_2SO_4) has been well known as a good source of nutrients for many vegetative crops since it contains the two elements potassium (K_2O) and sulfur (S) which are essential for plant development. While the potassium portion is associated with the movement of water, nutrients, and carbohydrates in

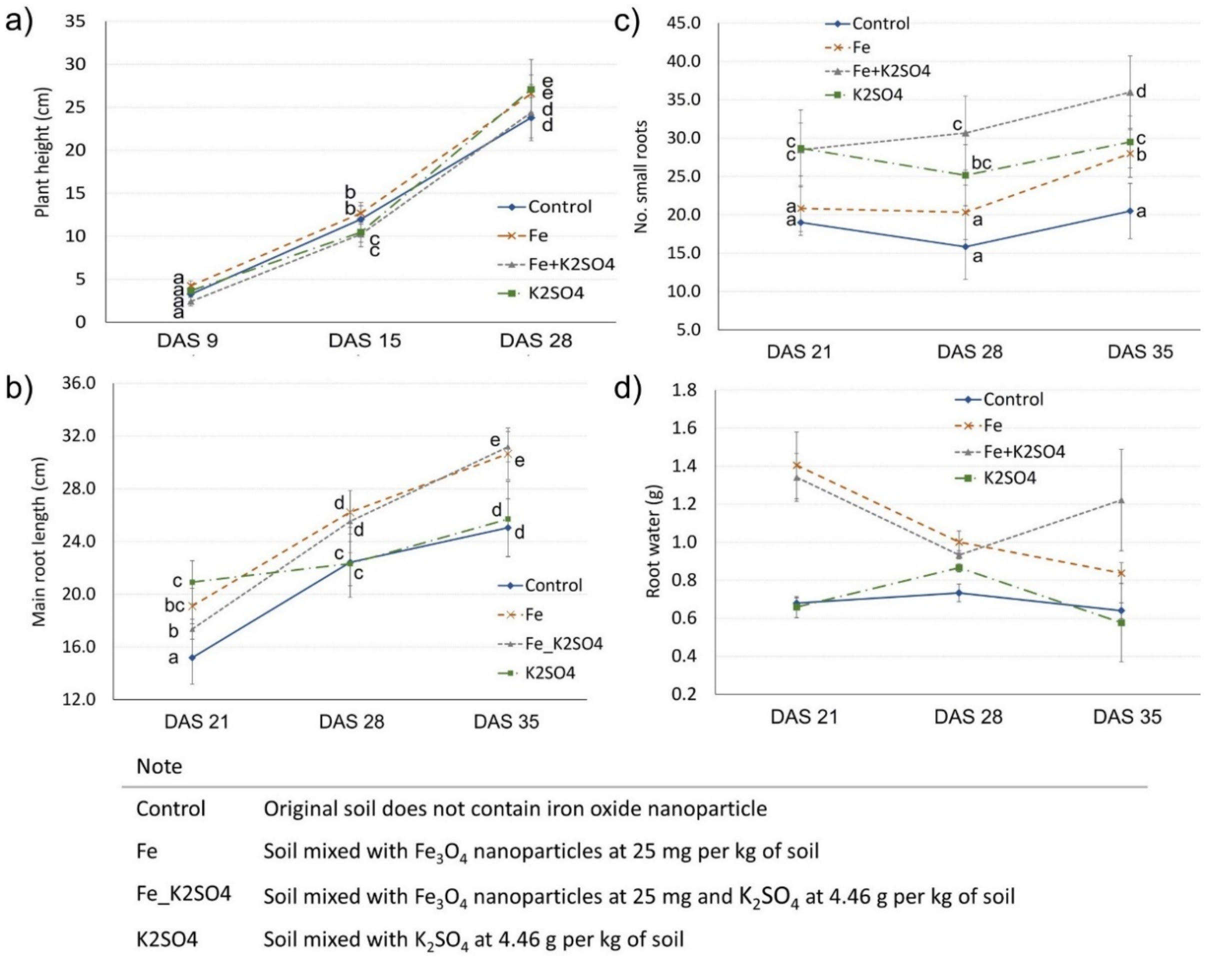


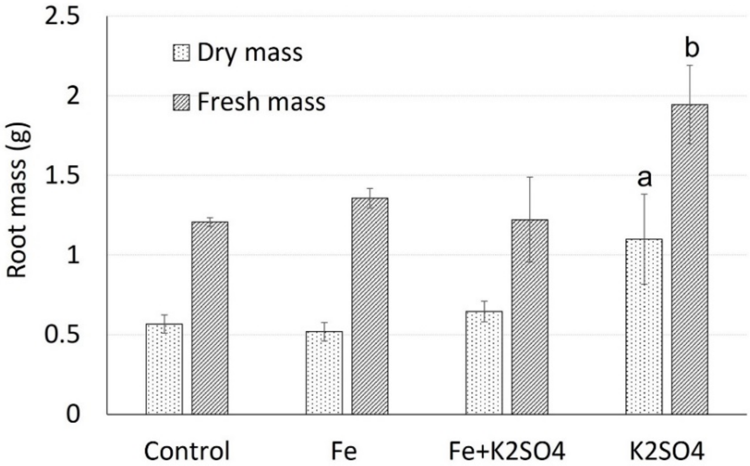
Fig. 2. Effects of iron oxide nanoparticles on the development of peas (*Pisum sativum*) under the presence of high potassium content in soil. DAS: days after sowing. Letter a, b, c, d, and e indicate significant differences (one-way ANOVA and Fisher LSD, $p \leq 0.05$).

plant tissue, the sulfur portion plays an essential role in protein synthesis and functions of enzymes. Because K_2O has a great effect on root development, differences in root metabolism and rooting patterns are of particular interest. The question is whether the effects of iron oxide nanoparticles in soil on plant growth are due to enhanced uptake of nutrients such as potassium of the plants or another cause induced from these nanoparticles? To answer this question an experiment to investigate the effect on plants of the iron oxide nanoparticles in the presence of potassium sulfate added to the soil was conducted. In this experiment, the soil was mixed with either iron oxide nanoparticles or both nanoparticles and K_2SO_4 for measuring the effects of the nanoparticles on peas development under the presence of K_2SO_4. The content of 4.46 g K_2SO_4 per kg of soil was calculated in order to double the K content in the soil.

Data in Fig. 2 indicates that all growth parameters of the plants in the control case show lower rates than those of the plants in the other cases. Under the presence of K_2SO_4 only in soil, the growth pattern of peas shows little difference to those in the control case. While the parameters of plant height and the number of small roots show a significant increase compared to those in the control experiment, the main root length shows the difference only in the early days, and from 28 days after sowing there is no significant difference. This finding confirms the important role of K_2SO_4 in the development of the root system in plants, especially in the early developmental period.

Meanwhile, plants in the two other cases having the presence of iron oxide nanoparticles in soil show significant increases in all developmental parameters compared to those in the control case. Especially, for plants in the case where the soil was mixed with both nanoparticles and K_2SO_4, a significantly higher number of small roots is observed compared to plants in all the other cases (Fig. 2c). The other developmental parameters of plants including plant height, main root length, and root water content in these two cases, in which the soil contains the nanoparticles, show no significant difference, but all are higher compared to those of plants in the two other cases with no presence of iron oxide nanoparticles. Once again this finding confirms the essential role of K_2SO_4 in root system development in plants. In addition, the iron oxide nanoparticles can also play role in the development of roots but might give priority to the direction where the main root extends for more water absorption

Interestingly, comparing root masses in different treatments at 35 days after sowing revealed that the iron nanoparticle alone, or in combination with K_2SO_4 in the soil, does not affect the root mass (Fig. 3). K_2SO_4 alone induces an increase in both the fresh and dry mass of the root. This could be an interesting finding because, in order to be able to grow the root system in terms of both size and more water absorption while maintaining constant biomass, the tissue structure of the roots may have to change. This will require further studies at the tissue and cellular levels of the plant to thoroughly understand this phenomenon.



Note	
Control	Original soil does not contain iron oxide nanoparticle
Fe	Soil mixed with Fe_3O_4 nanoparticles at 25 mg per kg of soil
Fe_K2SO4	Soil mixed with Fe_3O_4 nanoparticles at 25 mg and K_2SO_4 at 4.46 g per kg of soil
K2SO4	Soil mixed with K_2SO_4 at 4.46 g per kg of soil

Fig. 3. Effects of iron oxide nanoparticles under the presence of potassium sulfate in soil on the root mass of peas (*Pisum satvum*). Letter a and b indicate significant differences (one-way ANOVA and Fisher LSD, $p \leq 0.05$).

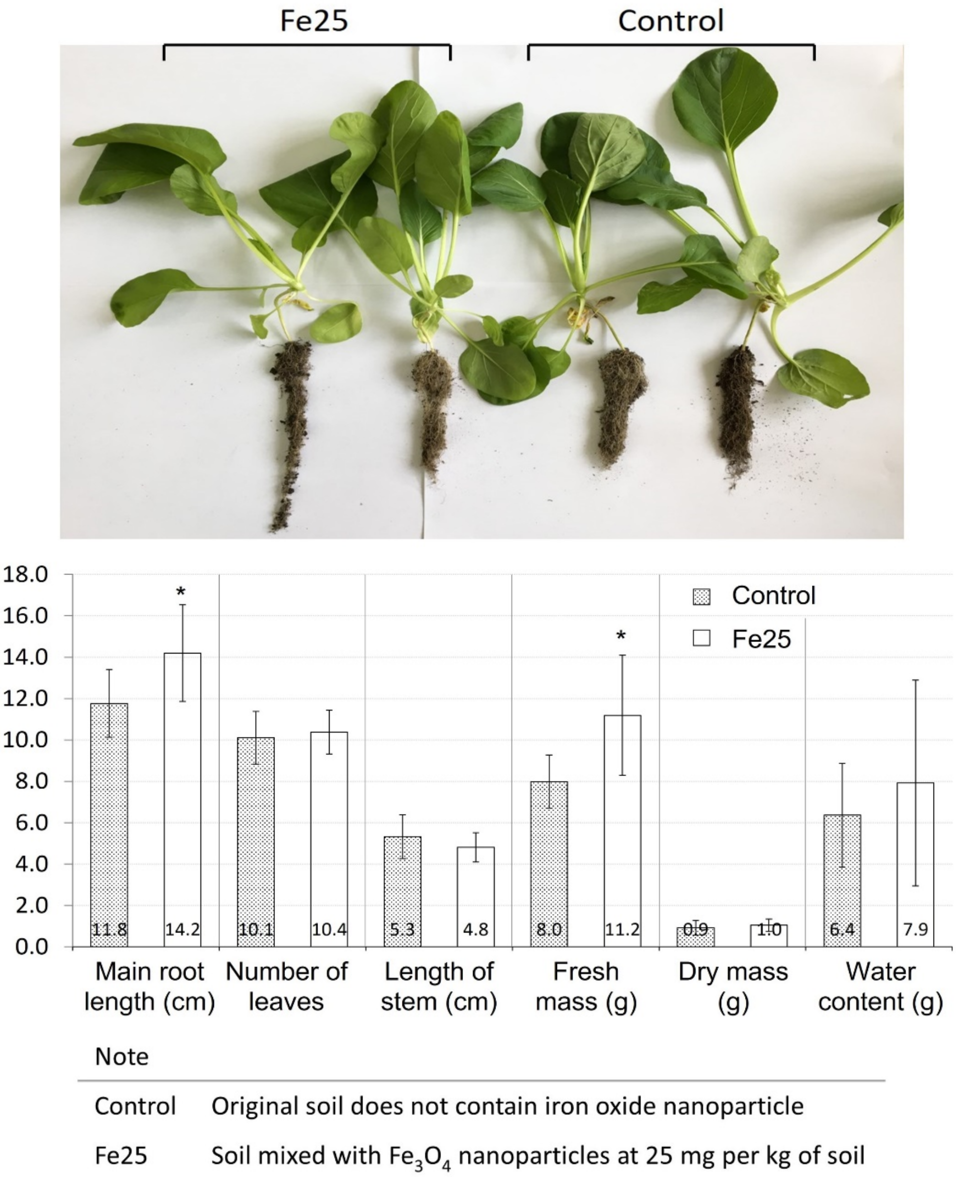


Fig. 4. Effects of iron oxide nanoparticles in soil on the developmental pattern of bok choy (*Brassica rapa*). * Indicates significant difference (one-way ANOVA and Fisher LSD, $p \leq 0.05$).

3.3. Iron Oxide Nanoparticles Stimulates the Development of Bok Choy (Brassica Rapa) in Term of Main Root Elongation and Increasing Water Absorption Capacity

In this experiment, the effects of iron oxide nanoparticles on plant development were investigated on another common vegetable, bok choy (*Brassica rapa*). Only one concentration condition which is 25 mg iron oxide nanoparticles per kg of soil was applied. The plant developmental pattern at 38 days after sowing was measured and shown in Fig. 4. Compared to plants in the control treatment where the soil contains no nanoparticle, those in the case where the soil is mixed with iron oxide nanoparticles reveal significant increases of the developmental parameters including the main root length and the fresh mass, while maintaining the dry mass. This developmental behavior is quite consistent with which observed in the experiments with peas (*Pisum sativum*). It once again suggests that the iron oxide nanoparticles in soil places positive effects on plant development and that these effects are in the direction of expanding the dimensions of the roots and increasing the ability of the plant to absorb water.

4. Discussion

The rapid development of nanotechnology and applications of engineered nanoparticles is creating new pressures on the environment. Nanoparticles, by their nature, when released into the environment, will have the ability to persist and accumulate over time, creating transformative effects on the environment and ecosystems. It is essential to understand the impacts of each type of nanoparticle on the ecological environment, human and animal health in order to provide appropriate recommendations and guidelines for use.

Iron oxide nanoparticles have long been considered safe and have been studied for use as a form of nano fertilizer for a number of crops [8, 11]. However, limited literature on the interaction between plants and iron oxide-based nanoparticles is available. An in-vitro study recently reported an enhanced root growth in several legume species including chick peas, green peas, and green gram when treated with low concentration iron oxide nanoparticle solutions. On a contrary, an inhibitory effect was observed in high concentration nanoparticle solutions [8]. It showed that the growth of peas root was inhibited when seeds were treated in a solution containing the iron oxide nanoparticles concentrations of ~ 27.7 mg/L. The hybrid Pt-decorated iron oxide nanoparticles even exhibited a higher inhibitory effect in comparison to the iron oxide nanoparticles. Note in solution that the seeds might be exposed more directly to the nanoparticles compared to when in soil. Other studies on soil pointed out an increase in root length in plants treated with iron oxide nanoparticles [7, 9, 11, 12]. These studies agree with the finding in this study that iron oxide nanoparticles at a moderate concentration in soil could place a positive effect on the root development of plants. The nanoparticles at high concentrations in soil could induce no effect or even inhibitory effect on the development of the root and plants. It should also note that this study figures out an enhancement in root development that is observed in both the main root elongation and the increase in the number of small roots. A study by Rizwan and colleagues (2019) reported on the increase in plant height of wheat treated with iron oxide nanoparticles [12].

In terms of application in environmental remediation, as mentioned above, iron oxide nanoparticles are being used quite commonly in many techniques. Among them, Phyto-fenton is a technique that uses a combination of iron oxide nanoparticles and vetiver plants to promote the decomposition of persistent organic pollutants such as the plant protection agent, DDT - Dichloro-Diphenyl-Trichloroethane, in the soil [4]. This technique requires the presence of iron oxide nanoparticles in addition to the presence of H_2O_2 produced in the soil by plants, especially from the roots. The growth of plants is therefore crucial to the success of this technique. To date, there have not been many studies on the physiological and molecular mechanisms induced by iron oxide nanoparticles on plant growth, as well as the interaction of the nanoparticles with other essential components and nutrients in plants. The results found in this study suggest that iron oxide nanoparticles may have a very different mechanism of action on root growth, in particular, and the whole plant growth, in general, when compared to the effects of nutrients in essential fertilizers for plants.

Finally, this study indicates that the plant dry mass (in the case with bok choy) and the root dry mass (in the case with peas) were not affected by the presence of iron oxide nanoparticle in soil, which is contrary to the study of Rui and colleagues (2016) [11] on peanut and the study of Rizwan and colleagues (2019) [12] on wheat, both concluded an increase in dry biomass in iron nanoparticles treated plant. This once again suggests that the physiological effects of iron nanoparticles on plants highly dependent on the plant species as well as the physical stage of the nanoparticles.

5. Conclusion

The study has measured the effects of iron oxide nanoparticles in soil on the development of peas (*Pisum sativum*) and bok choy (*Brassica rapa*). It showed that plant development is affected by the presence of iron oxide nanoparticles in soil mainly in terms of the main root length and the water content. At low soil concentration (25 mg/kg of soil) of the nanoparticles, the main root tended to elongate while remained the number of small roots at lower or not significantly different to those in control. Meanwhile, the water retention in root (for peas) and the whole plant (for bok choy) showed increased when seeds were sown in soil containing the iron oxide nanoparticles at the concentration of 25 mg/kg of soil. Further studies at the cellular level into the tissue structure and the inside effect mechanism would be needed in order to have a comprehensive understanding of the plant-iron nanoparticle interaction.

Acknowledgments

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