Effects of iron oxide nanoparticles on foliage development of red radish (*Raphanus sativus*) and bok choy (*Brassica rapa*)

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Abstract

Nanomaterials have been using in many applications covering many different aspects in life. Among nanomaterials, iron oxide nanoparticles (FeNPs) have been considered relatively safe, and appeared in number of applications from medical, health to environmental applications. This booming in using FeNPs leads to the appearance of them in different water bodies that might end up lately in plants and animals. This study reports on the effects of FeNPs on foliage development of two common vegetables, red radish (Raphanus sativus) and bok choy (Brassica rapa). It showed that radish was not affected by the nanoparticles regardless exposure scenarios. The developmental rate of bok choy, however, showed increased with FeNPs concentration in the scenario that seeds were pre-soaked in water before sowing on medium containing FeNPs. In the experimental scenario that seeds were soaked in solutions containing FeNPs before sowing on nanoparticle-free medium, it showed retardation in the developmental rate of the seeds.

Keywords: iron oxide nanoparticles, foliage development, radish, bok choy.

1. Introduction

Nanotechnology, the term defined by the National Nanotechnology Initiative of the United States as “Science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers”, was actually first pointed out by a famous American physicist Richard Feynman in 1959 at a meeting of the American Physical Society. As mentioned in the definition, the central object of nanotechnology is nanomaterials, which generally defined as materials with at least one dimension of the size of up to 100 nm and can be 3-dimensional particles of almost any shape [1]. Nowadays, nanotechnology has found its imprints in almost all aspects in life from science and technology including engineering, medicine, pharmacy, biology, agriculture and environment, etc. to manufacture of consumables such as cosmetics, sunscreens, surface coating materials, clothing and even some food products [2].

The blooming in applications of nanotechnology, as a matter of course, will associate with new risks. Number of reviews and reports have mentioned about the toxicity potency of nanomaterials on human and animal health at cellular, organ levels. One of the major concerns recently is the possibility that nanomaterials could end up in environmental bodies infesting drinking water sources harming the health of humans and animals as well as placing impacts on ecosystems. Among nanomaterials, iron nanomaterials of different forms could be found predominantly in various applications from medicine [3-6] to environmental sector [2,7]. It therefore could be more chance for iron nanomaterials to end up in the environment such as aquatic environment where they may easily find their way to plant cells and organs before going to human and animal bodies through food chains. In this study, using one kind of iron oxide nanoparticles self-synthesizing in our lab, we measure the impacts of the nanoparticles on foliage development of some common vegetables to point out which implication could iron nanoparticles, in particular, and nanotechnology, in general, place on our plant ecosystem.

2. Materials and Methods

2.1. Materials

The iron nanoparticles were magnetite (γ-Fe2O3) nanoparticles coated with Dimercaptosuccinic acid (DMSA) for surface stabilization which gives the particles negative surface-charged (Zeta potential: -39.1 to -41.3 mV) and sizes of around 10 nm in diameter (Fig. 1). The nanoparticles were synthesized and kindly provided by Sakakibara Laboratory at Waseda University, Japan. Murashige & Skoog Plant Salt Mixture (MSSM) commonly used for plant tissue culturing was obtained from Nihon Pharmaceutical

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Co., Ltd. Japan. Seeds of red radish (*Raphanus sativus*) and bok choy (*Brassica rapa*) were purchased from local providers: Dong Nam Viet Co., Ltd and Viet A Co., Ltd, respectively. The seeds were clean, pre-treated for anti-fungi and packed in closed tin bags.

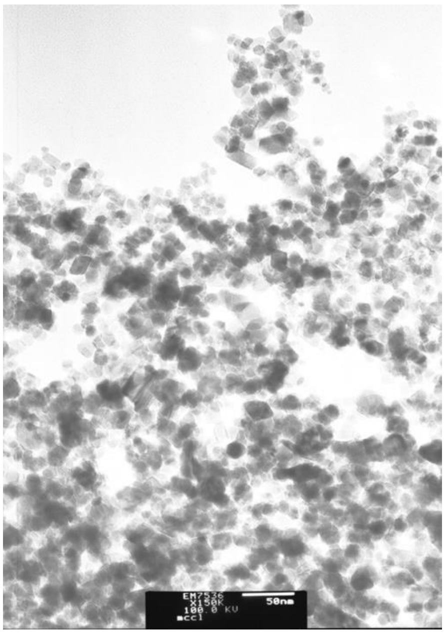

Fig. 1: TEM image of DMSA-coated iron oxide nanoparticles (100kV, JEM-1011, scale bar: 50nm)

2.2. Seed exposure scenarios

For in vitro trials, seeds of both types of vegetables the same size were carefully selected and rinsed with deionized water. There different scenarios were implemented:

- Scenario 1: Seeds were soaked in distilled water for 5h before sowing on MSSM/agar medium containing iron oxide nanoparticles (FeNPs) at different concentrations: 0 (control), 0.01, 0.001, 0.0005 and 0.0001 ppm in flasks. The FeNPs suspended medium was prepared by adding the particles into MSSM medium at different concentrations before sterilization at 110ºC for 20 min. After the sterilization, flasks were taken out and gently rotated few times to ensure equal distribution of FeNPs inside the medium before they became solid.
- Scenario 2: Seeds were soaked in distilled water containing FeNPs at different concentrations: 0 (control), 0.01, 0.001, 0.0005 and 0.0001 ppm, for 5h before sowing on nanoparticle-free MSBM/agar medium in flasks.

2.3. Experimental conditions

All experiments were implemented at ambient temperature between 24-26ºC, humidity of 80 ± 5%. Experiments were arranged in an out-door glass box which ensures all flasks to receive light from natural sunlight. All flasks were covered with cotton buttons which ensures freely diffusion of oxygen into the flasks.

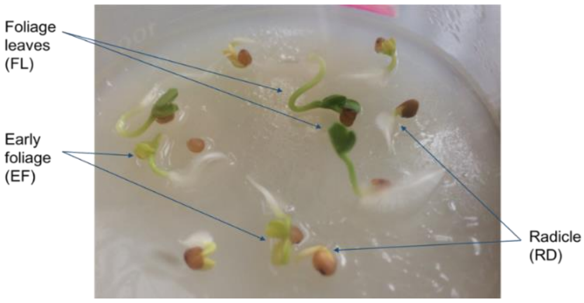

Fig. 2: Illustrations of the three stages of foliage development of vegetables.

2.4. Observation of foliage development of seeds

The foliage development of vegetables was divided into three stages: 1) radical (RA) indicates seeds sending out bugs (shoots) or under germination; 2) early foliage (EF) indicates the stage where foliage leaves have already formed but not completely turned into all green; 3) foliage leaf (FL) indicates the stage where foliage leaves have completely turned green. Figure 2 shows illustrations of the three stages of the foliage development. At certain time points after sowing, numbers of seeds presented in each stage were recorded for later calculations and analysis. The rate was calculated as the ratio of seeds in a certain stage (RA, EF or FL) of the foliage development to the total number of seeds sowed in each flask.

2.5. Data analysis

All experimental treatments were performed in triplicate, with untreated blanks to ensure quality control. Statistical analysis of data was performed with a StatPlus:mac LE v5.9.92 software (AnalystSoft Inc., USA) using one-way ANOVA and post-hoc t-test. Significance level was assigned at $p \leq 0.05$.

3. Results

3.1. Effects of DMSA-coated iron oxide nanoparticles on foliage development of red radish (Raphanus sativus)

In this experiment, radish seeds were exposed to negative-charged FeNPs followed the two exposure scenarios described in the Materials and Methods. At certain time points, number of seeds presented at different stages of the development as also defined in previous part was recorded and calculated for the rate of seeds in each stage. A statistical analysis with a

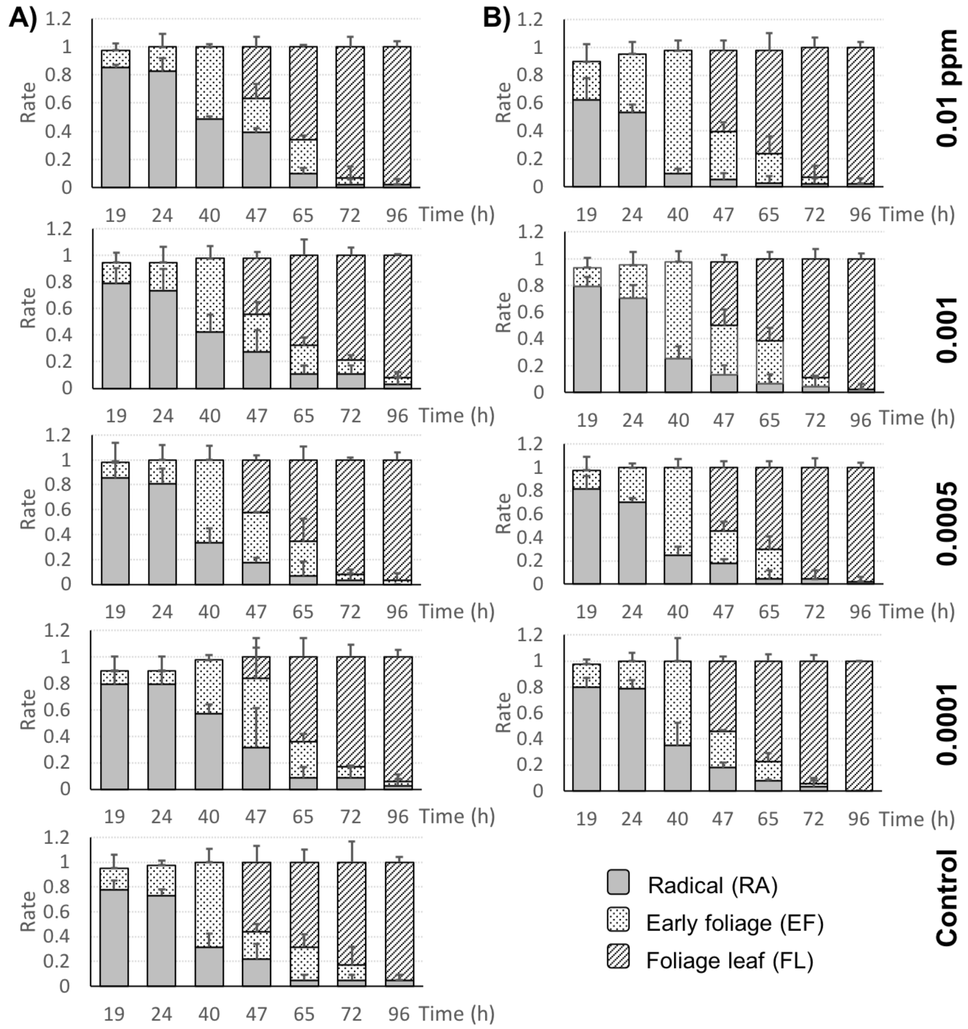

Fig. 3: Effects of FeNPs on foliage development of red radish (*Raphanus sativus*) at different exposure scenarios. A) Seeds were soaked in distilled water followed by sowing on FeNPs-containing mediums; B) seeds were soeked in water containing FeNPs at different concentrations before sowing on nanoparticle-free mediums. All data are presented as mean ± standard deviation

StatPlus:mac LE software with one-way ANOVA was followed at each time point to identify any significant differences. As revealed by the analysis, there is no significant difference between all treatment groups of each exposure scenario was detected (Figure 3A and B). This result indicated that the foliage development of radish seeds was not affected by the presence of FeNPs at different levels from 0.0001 to 0.01 ppm in the environment, regardless exposure scenario.

3.2. Effects of DMSA-coated iron oxide nanoparticles on foliage development of bok choy (Brassica rapa)

In the experiment with bok choy seeds, the seeds were also exposed to negative-charged FeNPs followed the two exposure scenarios. Data collections and treatments were also conducted the same way with that for radish seeds. Statistical analysis with a StatPlus:mac LE software with one-way ANOVA, interestingly, revealed some significant differences between different treated groups in both exposure scenarios. In the scenario 1 where seeds were soaked in water before sowing on FeNPs-containing MSSM/Agar medium, at high levels of the nanoparticles in the medium, the seeds showed faster transitions between different developmental stages (Figure 4A). At the concentration of 0.01 ppm of FeNPs, at 41 hours after the sowing, most of seeds already transitioned from radical (RA) stage to early foliage (EF) stage. It also pointed out at 47 hours after sowing that more seeds in the case of 0.01 ppm-FeNPs treatment were completed their transition into foliage leave (FL) stage. At this point, the case of treatment at 0.001 ppm of FeNPs also indicated significant number of seeds had been transitioned into FL stage. All seeds in the case of 0.01 ppm-FeNPs treatment completely

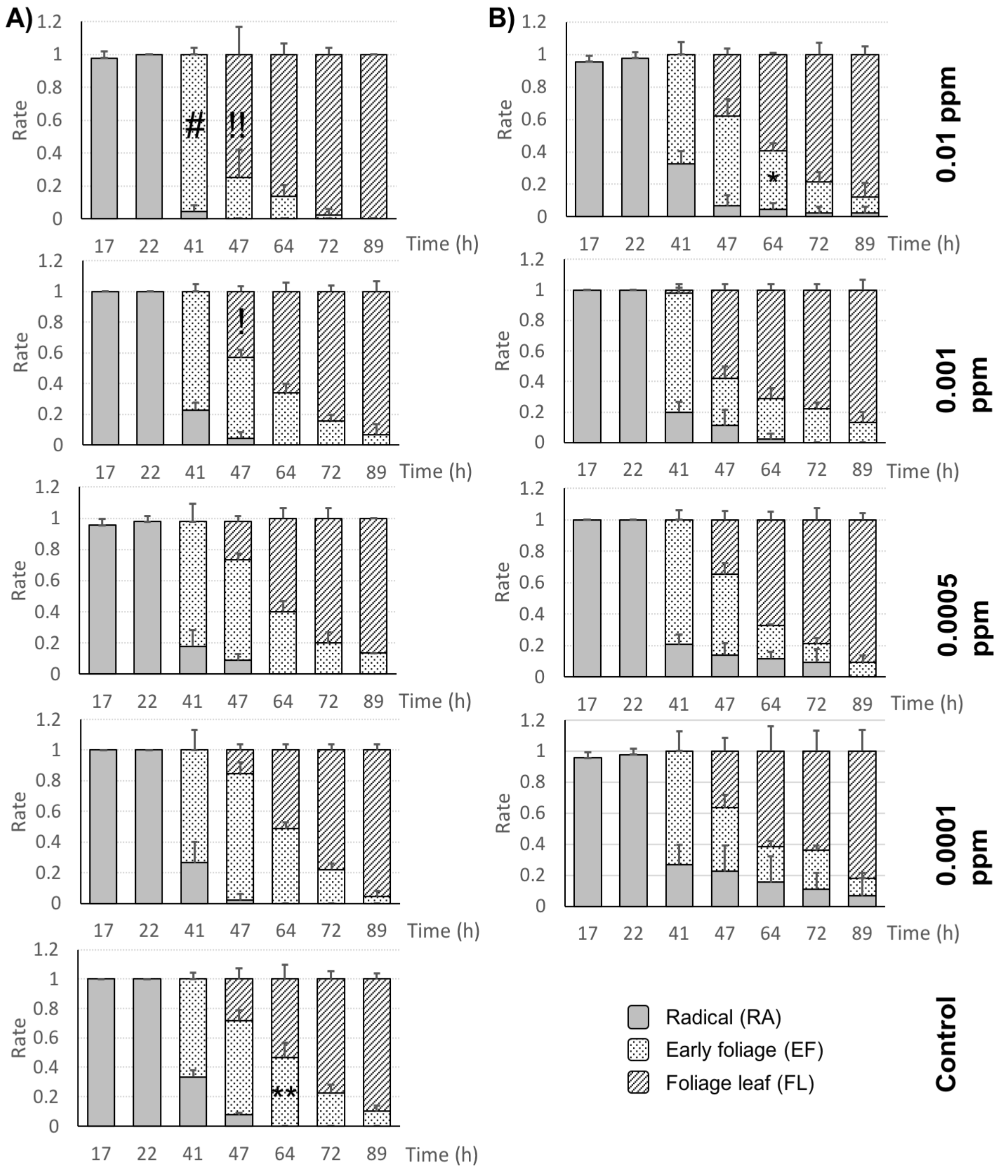

Fig. 4: Effects of FeNPs on foliage development of bok choy (*Brassica rapa*) at different exposure scenarios. A) Seeds were soaked in distilled water followed by sowing on FeNPs-containing mediums; B) seeds were soeked in water containing FeNPs at different concentrations before sowing on nanoparticle-free mediums. Marks: #, ! and * indicate significantly statistical differences. All data are presented as mean ± standard deviation

transitioned into FL stage, while in other cases, there were still some seeds under transition from EF to FL stages.

In the scenario 2 where seeds were soaked in solution containing FeNPs at different concentrations, the seeds, however, showed little retardations in transitions between different developmental stages (Figure 4B). At 64 hours after the sowing, less seeds in treated cases had completed their transitions into EF stage compared to that in control case. In addition, at this time point, in control case, all seeds had completed their RA stage and moved to EF and FL stages. This result showed opposite trend compared to the trend in exposure scenario 1, suggested that the effects of FeNPs on plants may different depending on stages of development and types of exposure.

4. Discussions

Although nanotechnology has found its root in almost all aspects in life, the environmental implications of engineered nanomaterials has just received great concerns from scientists and researchers in the field of environmental science and engineering within the last 10 years. Number of research articles have reported on the effects of nanomaterials on different ecosystems including plant ecosystems. However, results of nanomaterial impacts on plant development were often twisted [8]. Metal nanoparticles usually exert their toxicity over exposed plants through the generation and accumulation of reactive oxygen species (ROS) in plant tissues [8,9]. Toxicology of iron oxide nanoparticles has also been reported in number of studies. It has been showed that treatments with iron oxide (Fe_2O_3) nanoparticles can lead to reduction in seedling and root elongation in *Arabidopsis thaliana* [10]. Other studies, however, revealed positive effects of iron oxide nanoparticles on plant growth, such as the effects of γ-Fe2O3 nanoparticles on *Citrus maxima* plant via folia spray which reduce nutrition loss [11], or increase in leaf and pod dry weight, and grain yield in soybean [12]. A study by Rui and colleagues has revealed the role of iron oxide nanoparticles as promoting the growth of peanut by regulating phytohormone contents and antioxidant enzyme activity, thus suggested to use the nanoparticles as potential fertilizer for peanut (*Arachis hypogaea*) [13]. Some other hydroponic studies, however, indicated negative effects such as reduction in chlorophyll content and photosynthetic activity, plant growth harm, induction of oxidative stress and reduction in macronutrients uptake, etc. [8]. All together the effects of iron oxide nanoparticles on plants seem likely to be dependent on type of exposed plant, way of exposure and developmental stage of plant when being exposed.

5. Conclusions

In conclusion, this study has revealed the effects of DMSA-coated magnetite (γ-Fe2O3) nanoparticles on foliage development of red radish (*Raphanus sativus*) and bok choy (*Brassica rapa*). It suggested that red radish was not affected by the presence of the nanoparticles in both exposure scenarios, but bok choy with some increases in developmental rate. It should note that in this study, seeds were exposed to FeNPs at relatively low concentrations. The results indicated that the effects of FeNPs, in particular, and metal oxide nanoparticles, in general, in the environment on plants at different stages of their development could be either negative or positive. A study at molecular level to look deeply into the mechanism of action of the nanoparticles should be a future work.

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