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Effect of Plant Growth and Environmental Enhancement of Soils through Nanoparticle Application

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Abstract

Silver nanoparticles (AgNPs) have been manufactured in recent years and widely used in various fields. Reactive oxygen species (ROS), which occur in AgNPs, destroy cell membranes. It is widely accepted that ROS generated in this manner inhibit microorganisms growth and causes toxic effects, However, it does not affect cell membranes directly but positively affects growth in plants with cell walls. The nanoball used in this experiment is a new material that generates ROS stably and is used in aqueous solution. Results of this study indicate a 30% increase in yield of Ginseng mixed with culture soil. The analysis of soil condition after cultivation showed that the possibility of repetitive cultivation in soil mixed with Nanoball was high. This suggests that Nanoball is an antimicrobial active material due to the microbial / extermination effect of pathogenic microorganisms. Therefore, there may be potential applications in agricultural cultivation sites as a repetitive cultivation technology that reuses soil.

Keywords Silver nanoparticles, ROS, Soil, Ginseng, Repetitive cultivations.

1. Introduction

In history, silver has been used in a variety of applications. In recent years, silver nanoparticles (Silver nanoparticles) have been used in industrial and therapeutic applications for a variety of applications, including diagnostics, antibacterial, thermal and electrical conduction, and metal-enhanced fluorescence optics applications [4]. Manufacturers are incorporating AgNP into consumer products such as antibacterial agents, washing machines, refrigerators, air conditioners, and AgNP water purifiers. In particular, it has been used as an antibacterial and antifungal agent in medicine because the reactive oxygen species (ROS) generated from Ag has been reported to be effective for burns, wounds and bacterial infections. In this century, the use of AgNPs has become more important than ever because of the increased antibiotic resistance of bacteria [6].

The use of AgNPs in plants has been known to enhance the efficacy and promote growth of antifungal agents. ROS from Ag are characterized as toxic, but their activity has been shown to produce positively affect growth in some species of plants [7, 13, 14]. Studies on the gene expression of A. thaliana have shown increased metabolic activity, glutathione biosynthesis, glutathione S-transferase and glutathione reductase gene expression when exposed to AgNPs compared to Ag ions [3]. Studies of Ricinus communis (castor bean) seeds exposed to AgNPs increase increased ROS production and associated antioxidant mechanisms. That is,

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it increases peroxidase and superoxide dismutase enzyme activity, which leads to an increase in phenol.

Increased phenols are synthesized in plants to protect against pathogens, and report that the generated ROS promote root growth to a certain concentration [12].

On the negative side, however, the exposure of silver nanoparticles to plant growth varies. This is because it affects plants, including the various properties of nanoparticles, including size, shape, coating, amount, application and experimental methods. When these nanoparticles enter the plant, they can be toxic at various stages, such as oxidative stress, cytotoxicity, genotoxicity, germination, root and shoot growth and development. The effects reported on seed germination using AgNPs showed that ryegrass, barley and flax were toxically affected by low concentrations of AgNPs, but not completely inhibited [1]. Germination inhibition in rye grass and barley increased slightly with increasing AgNP concentration, indicating that plants have resistance to AgNP penetration. When a small AgNP flows out of the aqueous solution, it is inhibited when absorbed into the plant, and the toxic effect is small when not absorbed into the plant. For example, 150 nm AgNP particles did not inhibit germination and growth inhibition at concentrations up to 100 mg.ml –1 in O. sativa (Asian rice) compared to 150 nm AgNPs and smaller 20 nm particles. However, the 20 nm particle AgNP size has a more toxic effect, which inhibits the growth, suggesting that small particles are absorbed into the plant and inhibit the growth [11].

AgNPs can produce potentially conflicting results depending on the plant growth environment (hydroponic cultivation medium, soil or acidic, alkaline soil). NP materials tend to react and interact differently when placed in various support environments. I purchased a material in AgNP in which silver molecules do not elute in water but react with water to release ROS. This experiment was conducted because monitoring the bioavailability during plant growth cycle by applying it to soil could be beneficial when studying ROS activity in plants and soil.

2. Materials and Methods

Ginseng shoot (noi-doo) were purchased from ginseng hydroponic media. Seeds were washed well with sterile water-treated midstream and soaked for 14 hours in midstream or Nanoball treatment maintained at $18 \degree$ C. Germination experiments incubated in the plate was treated with 14 hours and 30 minutes in 24 hours at a temperature of $22 \sim 24 \degree$ C and 9 hours 30 minutes was set to dark treatment. The germination test was carried out for 7 days while keeping the Nanoball submerged in water and shaking to exchange water with a 24-hour cycle.

The spherical meso silica product (Nanoball) used in the experiment was manufactured by D'Molon Korea and commercially supplied and supplied for the experiment. Nanoball 0.2% (1000g: 2g) was mixed with the brand name Barker, a gardening soil purchased from Seoul Bio, and applied to general plant growth experiments (Fig. 1).



Figure 1. Nanoball 0.2% (1000g: 2g) was mixed with the brand name Barker: A, Real size Nanoball which contained Ag. B, Nanoball mixed with soil.

3. Experiment and Result

Sterilized water and Nanoball treated water were used to supply the moisture needed for the plants to germinate. Dissolved oxygen (DO) refers to oxygen dissolved in water and molecular state in solution. Dissolved oxygen in pure water at 20 ° C and 1 atm atmosphere reaches saturation at 9 ppm. Dissolved oxygen decreases with increasing temperature and increases with atmospheric pressure.

The mechanism of ROS generation in nanoballs is that ROS is generated stably by the reaction of Ag contained in silica with dissolved oxygen in water. However, because the half-life is short, it is effective to use it immediately. Frequent replacement is effective. In general, ROS has an inhibitory effect on the growth of microorganisms. This experiment's purpose was to investigate the effects of dissolved oxygen and ROS generated rather than the effects of microorganisms in germination experiments (Fig. 1).

Soil diseases such as root rot and detrimental fungi are the main causes of crop disturbances and damage caused by these diseases is increasing every year. In particular, the root rot caused by ginseng cultivation has no control method and results in lower yield and quality due to early harvest. Ginseng producers are anxious about the occurrence of soil diseases, and the use of strong pesticides to disinfect soil in ginseng fields is increasing.

These practices affect soil microbiology and soil nutrient circulation, but producers are skeptical of the proper microbial restoration required for soil after soil disinfection. The reason is that it is difficult to acquire microorganisms that can adapt to the destroyed ecological environment, and the remodeled environment is difficult to fit the growth conditions. Natural recovery methods allow for a period of rest and recovery, but ginseng has a long period of cultivation. In order to demonstrate the effectiveness of ROS generated in nanoballs as an effective method for plant growth, AgNP nanoball generated ROS in a water solution was applied to soil used for ginseng cultivation. (Fig. 2B).



Figure 2. Effect of Nanoballs for plant growth during culture: A, magnified left part of B picture without Nanoballs, B, Comparison of Growth Status of Plants with and without nanoball C. Magnified right part of B picture with Nanoballs.

Considering that ginseng cultivation is the highest yield per unit area crop in South Korea, it was commissioned to farmers who cultivated and sold for 3 months as a vegetable. At this time, all provided nutrient solution was treated in the same way, and the growth rate was confirmed by seeding the same amount of germinated ginseng brain head in the same area. Comparisons were made between the treated area (Fig. 2C) and the untreated area (Fig. 2A). As a result, cultivation yield was increased by more than 30% in the treated area in a short period of time and there was a significant difference in the development status of each individual (Fig. 3 A). Cultivated ginseng grown by mixing nanoballs in the soil was healthy and well grown and did not

die (Fig. 3).



Figure 3. Comparison of Growth Status of Plants with and without nanoball. Effect of Nanoballs for plant growth during culture : A, without Nanoballs. B, with Nanoballs

With the recent increase in ginseng cultivation area, there is an absolute shortage of grassland with high ginseng cultivation efficiency due to rising land prices in South Korea. There are factors that make repetitive farming difficult. There are problems such as the increase of pests, the increase of cultivation management costs, the increase of costs due to the movement of facilities, and the difficulty of installing the latest facilities. There is an urgent need to establish countermeasures against root rot, which is a repetitive problem for soil. Therefore, it is important economically to solve the ginseng cultivation and arable land problem of soil restoration ability. The soil was visually inspected following cultivation. Soil treated with nanoball did not produce green algae and significantly reduced the fungus species (Fig. 4). This suggests that the nanoballs used in our experiments are consistent with previous reports, which are known to have a toxic effect on microorganisms due to the generation of ROS in aqueous solutions that are reliably supplied in soil [6]. This is thought to be possible as a field grafting technology for sustaining the soil disease suppression effect and minimizing the pathogens of the ginseng cultivated soil. It is also an applied technology that can determine whether ginseng can be grown without increasing cropland.



Figure 4. Soil condition after plant culture without Nanoballs: A, with B, with fungus and green algae

The easiest way to determine the reactions that cause ROS is to directly measure root development. There was a significant change in the growth rate of about 30% depending on the presence or absence of nanoballs (Fig. 5A, B). These results may have relative effects as the growth of microorganisms decreases [2, 5]. However, the effect of root growth may be a greater factor due to the ROS. As several reports have reported that ROS promotes root development, it is likely that stable ROS generated in nanoballs had an effect [8]. In

order to investigate the effect of ROS from nanoball on plant growth, it is more efficient to examine the effect of ROS on the whole plant by chemical effect of ROS, rather than the signal mechanism reaction formed by direct contact and stress on the root. The ROS do not affect plant cells, but the experimental results show that it is not only effective for root length growth but also for entourage development. The reason for this is that many researchers report that ROS plays an important role in root hair, root tip, and development [8, 9, 10].

In order to determine the possibility of repetitive cultivation, the soil that was difficult to cultivate was reused (Fig. 4B), and experiments were conducted to find more obvious effects by mixing AgNP nanoballs. As a result, the green algae disappeared, restored to normal soil, and the growth of plants was normalized (Fig. 5C), showing the possibility of repeated use of the land.



Figure 5. Typical plants during soil culture with Nanoballs(A)and without nanoball(B). Plants grown by applying nanoballs for soil used without nanoballs(C).

4. CONCLUSION

Free radical species (ROS), which occur in AgNPs, destroy cell membranes. The ROS generated in this way have a positive effect on growth without directly affecting the cell membrane in plants with cell walls. It acts as an antimicrobial agent to microorganisms that cause root rot and cause positive effects on ginseng culture. In order to verify the effect of ROS generated from this material on plant growth, there was a 30% increase in the cultivation of Ginseng mixed with culture soil. The analysis of soil condition after cultivation showed that there is a high possibility of repeated cultivation in soil mixed with nanoballs. It is considered that the technology can be applied at the agricultural cultivation site by utilizing the possibility of reusing soil.

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