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Investigation of the Role of Brassica Roots & Fusarium Mycelium in Accumulation of Gold Particles

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A B S T R A C T

In the last decade, the production and use of metal particles in the nanoscale spread are due to the latter efficiency in solving many obstacles in various fields of science, engineering, agriculture and medicine. Gold particles in the small scale show catalytic, magnetic, electrical, mechanical, biological and chemical properties due to the high surface-to-volume ratio where appearance of these particles interact and move higher in properties and strength. Several studies have obtained nano-particles of several types of metals such as silver, copper and gold from plants and microorganisms. The current study, thus, aimed at finding out the ability of the mustard plant root to collect gold particles(Au), as well as the mycelium of the fungus *Fusarium oxysporum*, where the examination technique using the scanning electron microscope (SEM) was used to confirm the presence and accumulation of gold particles. It is vital using the biological system of the organism. This method is environmentally and friendly does not result in toxins or high use of energy. It is characterized by its low cost and high efficiency in obtaining results. Three different concentrations of colloidal gold solution were used in the current study 2, 4, 6 ml, which produced a concentration exceeding 6 ml. In deposition of the largest percentage of gold in each of the plant root and fungal hyphae.

Introduction

The synthesis of metal particles of all kinds using biological sources can eliminate the problem of pollution by using chemical or physical methods (Soltani Nejad *et al*. 2015) The use of biological sources such as plants and its extracts to synthesize gold particles has become a common technique in recent studies as well as the use of microorganisms such as bacteria and fungi (Khatami & Pourseyedi, 2015; Soltani Nejad *et al*. 2015), that the mechanism of deposition of gold particles in plants occurs through the reduction of metal ions through sugars in root cells (Khatami *et al*. 2015), that the manufacture of particles and their deposition in plant parts with this biological technique has advantages as it is environmentally friendly and low cost in addition to the speed of its formation (Kumar, 2019). In addition, the mustard plant has a great economic importance in some countries for its food uses and achieving food security for the peoples of those countries (Kumar, 2019) where the mustard plant belongs to the Brassicaceae family and the genus B. Juncea as well as the genus Sinapis arvensis. There are some minerals absorbed in the soil with slow movement and unable to reach the root zone (Dhaliwal *et al*. 2020b). It was found that plants of the cruciferous family and mustard plants have the ability to absorb minerals from the growth environment by making complexes with metal ions in the presence of the natural Ethylenediaminetetraacetic acid (EDTA) inside the plants or the industrial one added to the growth medium as it helps to precipitate minerals inside the roots. The tolerance of plants to the absorption of metal ions differs according to the different plant species and genotypes of those plants. Besides, many plants of the cruciferous family have the ability to grow in a medium with a high concentration of heavy metals and complete their growth period without being affected by the concentrations of those metals (Baker *et al*. 2000 and Setia *et al*. 2020b). The factors that affect the mechanism of mineral accumulation in plant roots depend on several factors including the type of mineral, the extent of plant uptake and how the mineral is transported to the plant (Baldantoni *et al*. 2014)and the direct contact with metal ions (Bauddh & Singh 2010). Particles are defined at the nanoscale and are defined as the dispersion of particles of solid particles with at least one dimension in the size range (10-1000) nm (Thakkar *et al*. 2010 and Mohanraj & Chen 2006). The most important feature of particles is their surface area to aspect ratio. This allows them to interact with others more easily (Narayanan *et al*. 2010 and Thakkar *et al*. 2010) as well as having unique thermal, optical, physical, chemical, magnetic and electrical properties (Husseiny *et al*. 2007 and Durán *et al*. 2007). There are organisms that have developed a particular resistance to a certain number of metals and are capable of synthesizing more than one type of mineral particle using the organism. The synthesis of "natural" biomineral particles can be divided into two parts. The first is bioreduction in which metal ions are chemically reduced to more biologically stable forms;i.e. Enzyme. The second section is the bio-absorption. It is by withdrawing metal ions from the aqueous medium or soil (Deplanche *et al*. 2010) by the microorganism (mushrooms) by the mycelium cell or the spinning cell wall. Some types of fungi and plants form peptides in the cell wall forming a complex component. With the metal ion, this helps to precipitate metal particles on the cell surface or cell wall (Yong *et al*. 2002). Fungi have been used in the production of mineral particles because they provide special advantages for particle manufacturing as the fungal hyphae help to precipitate a larger amount of metal to increase its surface area (Mukherjee *et al*. 2001). And because fungi secrete has more amounts of protein than bacteria, this leads to increased particle sedimentation (Rai *et al*. 2009). *Fusarium oxysporum* was used in several studies dealing with particle synthesis. 15.5 nm with the help of thread proteins (Ahmad *et al*. 2003). Previous studies were also conducted to assess the extent of mineral absorption by the cruciferous family. And these studies were based on the analysis of plant biomass and vegetative parts only (Dhaliwal *et al*. 2020a and Wu *et al*. 2004). Hence, this study aimed at evaluating the efficiency of the root system of the mustard plant and the fungal hyphae of the genus *F. oxysporum.* The adsorption and accumulation of gold particles are under multiple concentrations of colloidal gold.

Materials & Methods

Collection of Mustard Seed

The wild variety mustard seeds were locally obtained from the Iraqi Seed Production Company in Mosul, while the seeds of the Indian variety were purchased from the Turkish market for the sale of crop seeds.

Cultivation of seeds in soil and hydroponic medium:

The two mustard cultivars wild (Sinapis arvenis), Indian(Brassica junceae) were grown in the canopy in poles after 10 days germination occurred in the two cultivars, and the researcher continued to water them for a period of 30 days until the seedlings reached the appropriate size. .

Growing of hydroponic medium with colloidal gold.

A seedlings was taken of the active mustard cultivars wild (Sinapis arvenis), Indian (Brassica junceae) , cultured on medium in flasks and colloidal gold was added at concentrations (2,4,6) ml/50 ml of medium by three replications.

Detection of gold particles accumulated in mustard roots

After drying the plant samples, they were taken to the laboratory to detect gold particles (AuPs) using a scanning electron microscope (SEM) at a wave length of 3000KV by taking a section of the root with a diameter of 1 cm from both cultivars and placing it on a transparent disk with a sticky face and pressing with another cover, then the sample is placed in the device scans the sample at a voltage of 100 kV (Nagar *et al* 2016) with several magnifications until AuNPs were reached.

Collection of fungal Sample

A number of fungal isolates were obtained from the soil of the fields of the Agricultural Technical College / Mosul as they were isolated according to the method of Cappuccino and Sherman (1996).

Isolation and identification of *F. oxysporum*

After purifying and identifying a number of growing fungal isolates, the *Fusarium oxysporum* was isolated from the soil selected for its positive result and its possession of oxidation enzymes and its use in the synthesis of gold particles as the fungus was activated in Petri dishes containing PDA.

Growing *F. oxysporum* on peptone-yeast-glucose (PYG) liquid medium with colloidal gold.

A disc was taken from the edge of the active colony of *F. oxysporum* isolated and cultured on PYG liquid medium in glass flasks, and colloidal gold was added at concentrations (2,4,6) ml/50 ml of PYG liquid medium by three replications.

Detection of gold particles in mycelium parts

After the growth of colonies and the formation of fungal threads, the medium was filtered by Whatman No1 filter paper to obtain the fungal biomass. Then, the wet weights were taken and placed in a drying oven to obtain the dry weight of the biomass. After the fungal samples were dried, they were taken to the laboratory to detect gold (Au) particles. Using a scanning electron microscope (SEM) at a wavelength (HV3000) by taking sections of fungal filaments and placing them on a transparent disk with an adhesive face and pressing with another cover, then the sample was placed in the device and the sample was scanned with a voltage of 100 kV (Nagar *et al*., 2016) with several zoom in until getting the shape and size of the gold particles.

Statistical Analysis:

The experiment was carried out according to a completely randomized design (CRD) with three treatments and three replications. The results of the experiments were statistically analyzed after arranging and tabulating them using the statistical analysis program (SPSS) according to the analysis of variance (ANOVA) test. The averages of the treatments were compared using the Revised Least Significant Difference at the probability level of 0.05 according to (Obaid, 2022).

Results & Discussion:

Detection of gold particles accumulated in mustard roots

After drying the samples of the mustard plant, the root zone was cut off and taken to the laboratory for examination using (SEM) technology to detect the accumulated gold particles. The root zone, where the highest percentage was at a concentration of 6 ml for the wild variety with a rate of 1.9%, followed by a concentration of 4 ml, was compared to the Indian variety as in Table 1. The barley plant grown on aqueous medium was used and added at concentrations of gold nanoparticles, which resulted in changes in the chemical composition of the cell wall depending on the physical and chemical properties of AuNPs, i.e. their size and surface charge. As a result, an ionic exchange occurred between the root and the nutrient medium, which led to the withdrawal of gold nanoparticles, and this is one of the adaptive responses Plants have mineral orientation, which is a change in the chemical composition of the cell wall, as found in (Xiong 1999).

Table 1. Percentages of gold particles accumulated in the root zone of the mustard plant.

Varaity	Concentrations/ml	Au%
Brassica junceae	2	0.4
	4	0.9
	6	1.5
Sinapis arvenis	2	0.5
	4	1.2
	6	1.9

Measurement of gold particle sizes in the root

The diameter of the nanoparticles in the (SEM) examination images was determined by relying on the image scale using the (Image j) program where the image scale was determined and included in the program, and then the particle diameter was measured in the images by determining the distance or diameters by means of the indicator and then the diameter was calculated. The particles within certain directions in the program, and the sizes of the gold particles were obtained. As the particle sizes ranged between 424-1222.82 nm in the Indian variety, while the wild variety, the sizes ranged between 99.52-1699 nm as in Table 2 and Figure1 . Also, Rodriguez *et al*. (2007) and Gardea-Torresdey *et al*. (2005) found gold nanoparticles in the roots of different plants with sizes that differ depending on the type. Plant and concentrated gold solution, Barrena *et al*. (2009) found the formation of AuNPs in the dry biomass of tobacco plants with sizes between 2-52 µg. Likewise, Zayed & Eisa (2013) found the accumulation of gold particles in the leaves of some plants with sizes ranging from 32-54 nm at low concentrations of gold chloride, either at using high concentrations of HAuCL4. This was consistent with what resulted from concentration 4 and 6 mL.

Table (2) Sizes of gold particles in the root zone of the mustard plant

Varaity	Concentrations/ml	gold particle size (nm)
Brassica junceae	2	1222.85 ±728.21
	4	221.20 ±89.02
	6	424.00 ±188.13
Sinapis arvenis	2	936.23 ±575.60
	4	99.52 ±4.10
	6	1699.92 ±769.12

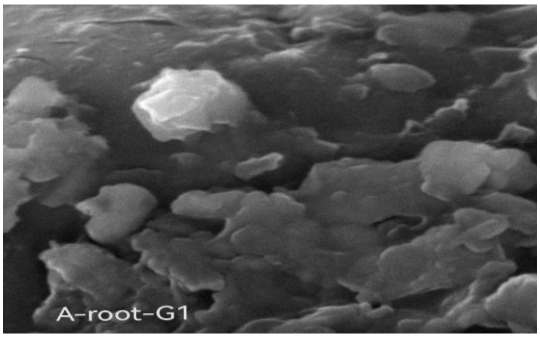

Figure (1). SEM images of gold particle on the root of *Brassica junceae*

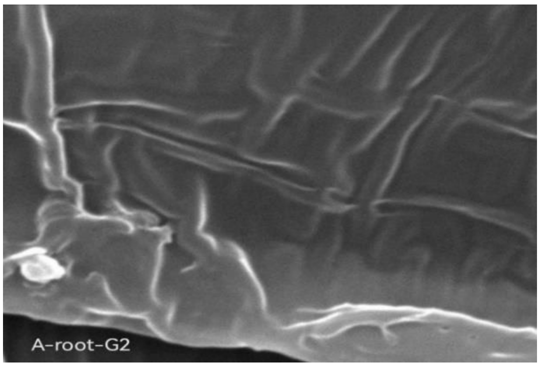

Figure (1). SEM images of gold particle on the root of *Sinapis arvenis*

Detection of the proportions of gold particles in the fungal hyphae:

After growing the *F.oxysporum* isolated in PYG liquid medium, the biomass of the fungus was filtered using Wattman No.1 filter paper, and then parts of the fungal filaments were taken for examination to detect the accumulation of gold particles. SEM technology was used for the purpose of detection. As the results of Table 4, Figure2 showed that obtaining the percentages of gold particles at a concentration of 6 ml, at a rate of 4.2% was followed by a concentration of 4 ml, then 2 ml (Coleine *et al*. 2021, Faghri zonooz *et al*. 2012). The reason was attributed to the interaction of the fungus F. oxysporum with a high concentration of added colloidal gold, which led to a rapid bioaccumulation of gold particles (Coleine *et al*. 2021). Gold at the nanoscale stimulates basic reactions in the organism such as metabolism, oxidation and reduction. And through the metabolic interaction carried out by the fungus *F. oxysporum* in the environment in it can filter Au particles from the medium and precipitate it in mushroom hyphae (Chen & Rozhkova, 2018 and Das and Marsili, 2010, Gupta *et al*. 2011, Kitching *et al*. 2015, and Reith *et al*. 2007)

Table (3) Percentages of gold particles in the fungal hyphae of *F. oxysporum*

Fungal	Concentrations/ml	Au%
F. oxysporum	2	1.7
	4	2.3
	6	4.2

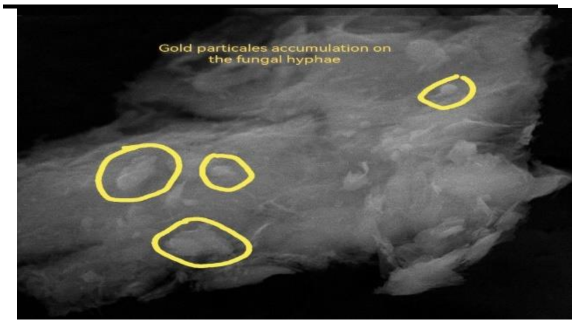

Figure (3) SEM image of *F.oxysporum* showing presence of gold nanoparticles on mycelia

The plants were unable to grow in harmful media with a high concentration of minerals. Thus, this study demonstrates the ability of the mustard plant to develop certain mechanisms in the root system to tolerate and adapt to different concentrations of colloidal gold. Ion exchange with the surface of the root cell wall, which has a negative charge, then oxidizes it by proteins, sugars and enzymes, and then reduces it to remain inside the root and accumulate, as well as the fungus Fusarium oxysporum proved its ability to withdraw gold and deposit it on the fungal spindle. The production of gold nanoparticles by the biological method ("green synthesis") is environmentally friendly and allows to reduce the amount of harmful and toxic chemical by-products (Coleine *et al*. 2021;Rossi *et al*. 2002 ; Meyers *et al*. 2008).

Conclusion:

The technique of detecting gold particles in the root of the mustard plant and the fungal mycelium of F. oxysporum was not clear, and most of the previous studies relied on processing outside the biomass of the plant and fungus, such as using plant extracts and also the biomass extract of the fungus. In this study, it was achieved to manufacture gold particles within the biomass of plants and mushrooms depending on the natural oxidation and reduction process carried out by the plant with the help of certain enzymes, including the EDTA enzyme, as well as sugars and proteins present within the plant cells, which led to the accumulation of particles in the root of the mustard plant in varying proportions when different concentrations, where the high concentration (6 ml) of colloidal gold, showed an accumulation of 1.9% of gold in the root compared to the Indian cultivar with a percentage of 1.5% . As for the percentages of gold accumulated in the fungal hyphae, the higher concentration exceeded 6 ml by 4.2%

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