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Detection of gold particales in *Fusarium oxysprum* and Leaves, Stems of *Brassica junceae* and *Sinapis arvenis*

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A B S T R A C T

The current study aimed to reveal Gold (Au) particles in the mycelium of *Fusarium oxysporum*, isolated from the soil of the fields of the Agricultural Technical College, Mosul, as well as in the vegetative parts of the Indian and wild variety of *Brassica junceae* and *Sinapis arvenis*. At a concentration of 6 ml and a size of 20.90 nm. As for the two cultivars of the mustard plant, 0.2% gold particles were found in it, while the leaves of the wild variety did not have precipitation of particles, and the particle size ranged from 20.90-776.73 nm. Particles were detected using a scanning electron microscopy (SEM) technique and an EDX meter.

Introduction

The technology of manufacturing gold particles is a science, engineering and technology that is measured on the nanoscale (1-100 nanometers) and because of the chemical property of these particles. They are not observed in normal sizes. In recent years, the manufacturing of these materials has accelerated, and the manufacturing techniques and materials used for manufacturing have differed and entered the industrial and engineering fields [1]. There are different pathways and methods for synthesizing these particles, including chemical and physical, and they are often expensive and need heat and certain processes in manufacturing. Therefore, many researchers in manufacturing turned to biological methods, being inexpensive and environmentally friendly, i.e. preparing particles from plants, as they are simple and available products. It does not cause environmental pollution [2], [3], [4], [5], [6], [7], [8], [9]. Gold is considered an inert element in nature and is characterized by excellent characteristics such as high free electron density, malleability and conductivity. However the small nanoparticles are made from it that enter into many applications and are modifiable such as diagnostics, bioimaging, transport of therapeutic substances. It is considered a catalyst for enzymes, as well as cleaning the environment from pollutants [10], [11], [12], [13], [14], [15], [16], [17], [18]. Plants and microorganisms are heavily involved in the biosynthesis of particles, especially gold. Among the microorganisms that are able to synthesize particles are fungi, bacteria [19]. Microorganisms were used as tools for treating toxic substances by reducing the ions of the materials and then collecting those ions in the form of An element by enzymes produced by these microorganisms. It is noticeable that most types of fungi have been used to biosynthesize nanoparticles, whether inside or outside the living cell, through the preparation of certain extracts [20]. With reduced metal ions and then deposited in the fungal filament, these factors determine the shape and size of the manufactured particles [21]. The genus *F.oxysporum* , which was Introduced by the scientist Link in 1809, is considered one of the economically important fungal genera, as it includes many pathogenic species for humans, plants, and domesticated animals. It is produce many toxins such as Trichothecene and Zearalenone [22], [23], [24], [25], [26], [27]. The fungus reproduces asexually by Macroconidia and Microconidia. Sexual reproduction by production of ascospores [28]. The genus Fusarium is found in most parts of the world, mainly in soils [29]or in plant remains [30][31] in the form of an epiphyte or in plant tissue[32]. Brassica is a plant belonging to the Brassicaceae family, which includes several genera and varieties, including Indian mustard (Brassica juncea) and wild mustard (sinapis arvensis). [33], [34], [35], [36], [37]. The seeds, leaves, and oil are used in many medicines, including anti-tumor drugs (bendamustine), as well as in the treatment of rheumatism and joint infections, and it has pharmacological properties in heart disease and diabetes[38]. Recently, the effect of gold nanoparticles on living plant systems has been studied to see its ability to extract minerals from their environment and collect them in its parts [39], [40], [41]. Thus, it is included a study on the mustard plant, which proved its ability to accumulate gold nanoparticles in its biomass. Because it has characteristics that help it to do so, including its rapid growth and its ability to adapt to different environmental systems, as well as its large size and large leaf area [42], [43]. In a study [44] it was shown that the process of particle accumulation in plant parts takes place through ion exchange, where metal ions are withdrawn by the roots after oxidation and transfer to the stem and leaves by the upward copying process through the wood. Then they are reduced in the plant parts and become a zero-valence metal. Limited information is available on the effect of gold nanoparticles on plants. Hence the aim of this study is to detect gold nanoparticles in the fungal hyphae and shoots of mustard plants.

Materials and methods:

Plants and fungus were used to detect gold nanoparticles using techniques and methods as shown below:

culture media

a. *Potato Dextrose Agar (PDA). Prepare the medium according to the manufacturer's instructions (HIMEDIA).*
b. *Peptone-Yeast extract-glucose (PYG). Prepare the medium according to the manufacturer's instructions (HIMEDIA).*

Solutions used:

1. *colloidal gold 40 ppm/100ml (Turkey/Kozteks).*
2. *Safe Nutrition Addition Program (SNAP) Aqueous media solution by adding 20 μM to each beaker.*
3. *(TMB) 3,3',5,5'-tetramethylbenzidine.*

The source of the fungal isolate:

F. oxysporum isolate from the soil of the fields of the Agricultural Technical College / Mosul for the season 2021-2022, as it was isolated according to the method of [45].

Identification of fungal isolates:

The fungal isolates were microscopically identified using the Lactophenol Cotton Blue mounting method by observing the spores, hyphae and mycelium. [46].

Fungal oxidative stress test:

The ability of a number of growing fungal isolates to produce oxidative enzymes was tested according to the method [47].

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

The biomass of *F. oxysporum* was obtained after growing it on (PYG) liquid medium containing colloidal gold at concentrations of (2,4,6) ml, where the resulting biomass was taken using Wattman No.1 filter paper.

Detection of gold nanoparticles in fungal mycelium:

Gold nanoparticles (Au) were detected using a scanning electron microscope (SEM) at a wavelength (HV3000) by examining the fungal filaments, which are placed on a transparent disk with an adhesive face and pressed with another cover. Then the sample is placed in the device and the sample is scanned with a voltage of 100 kV [48] with several agnifications until reaching the shape and size of the gold nanoparticles.

Hydroponics:

Random samples of brassica plants grown in the shade were taken 30 days after germination, with an average length ranging between (15-20 cm), to the laboratory. The roots were cleaned of suspended dust and placed in flasks containing 50 ml of tap water for five days to acclimatize them to hydroponic conditions [49]. A complete randomized design (CRD) with three replications and four levels of measurement set and colloidal gold concentrations (2,4,6 mL) was used. 20 microliters of (SNAP) Safe Nutrition Addition Program (SNAP) nutrient solution was added

Detection of gold nanoparticles in stems and leaves of both mustard varieties:

After drying the plant samples, they were taken to the laboratory to detect gold nanoparticles (Au) using a scanning electron microscope at (HV3000) wavelength, by taking a section of the stem and leaves and placing it on a transparent disk with a sticky face and pressing it with another cover. Then the sample is placed in the device and it scans the sample with an effort of 100 kilovolts [48] with several magnifications until reaching the shape and size of the gold nanoparticles.

Measurement of gold nanoparticle sizes in stem and leaves of both mustard varieties:

The diameter of the nanoparticles in the (SEM) examination images is determined by relying on the image scale using the (Image j) program, where the image scale is determined and included in the program. Then the particle diameter is measured in the images by determining the distance or diameters by means of the indicator, and the diameter is calculated . The particles are within certain directions in the program, and the sizes of gold particles were obtained with sizes ranging from 20.10 - 1273.75 nm at the absorption of a wavelength of 50 µm.

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 analyzed statistically after arranging and tabulating them using the SPSS statistical analysis program 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 [50].

Results and discussion:

The ability of fungus to oxidize colloidal gold

After growing the F.oxysporum fungus, oxidation of the fungus occurred in the PDA growth medium, where the continuous color turned from white to blue, as in Figure (1). Protein and when a certain metal ion is present, it turns into an enzyme that works as a catalyst in the oxidation of the metal ion and then reduces it to a pure metal of zero valence [50], which stimulates the fungus to oxidize metal ions to different forms. Fusarium species secrete this enzyme and it is considered an efficient enzyme. High motivational [51].

Figure 1. Oxidation of *F.oxysporum*

Detection of gold particles in mycelium:

F. oxysporum was isolated from the soil of the fields of the Agricultural Technical College and was identified on the basis of morphological and microscopic characteritics. Gold nanoparticles were detected deposited on the fungal hyphae oxidized by *Fusarium oxysporum*. These nanoparticles were examined and diagnosed using the following techniques:

Examination using a Scanning Electron Microscope (SEM):

The results of this examination are a confirmatory result for the presence or absence of gold nanoparticles in the mycelium, and by observing the figure (2) provided by this device and the information it contains. It shows the presence of gold particles distributed sparsely in all parts of the mycelium and with high stability through the absence of clusters. There are many of these particles at a wavelength peak close to (160 nm) of the gold element, as well as giving information about the proportions of minerals present in fungi, Table (1), and this is consistent with what he found [47]. The concentrationof 6 ml gave the highest percentage of gold nanoparticles accumulated in fungal hypha 4.2 % compared with the concentration of 4 and 2 ml with percentages (2.5 and 1.7) %, respectively. The reason is attributed to the interaction of *F. oxysporum* with a high concentration of added colloidal gold, which led to a rapid bioaccumulation of gold nanoparticles [52]. Gold at the nanoscale stimulates basic reactions in the organism such as metabolism, oxidation and reduction. Through the metabolic interaction carried out by *F.oxysporum* in its environment, Au particles can be filtered from the medium and deposited in the fungus hyphae [53], [54], [55], [56], [57] that the mechanics by which the particles are manufactured is by the secretion of the fungal filaments of certain enzymes, including the NADPH enzyme, which are carriers with reduced metal ions and then deposited in the fungal filament, and these factors determine the shape and size Manufactured particles [58].

Table 1. Gold particles accumulated on *F. oxysporum.*

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

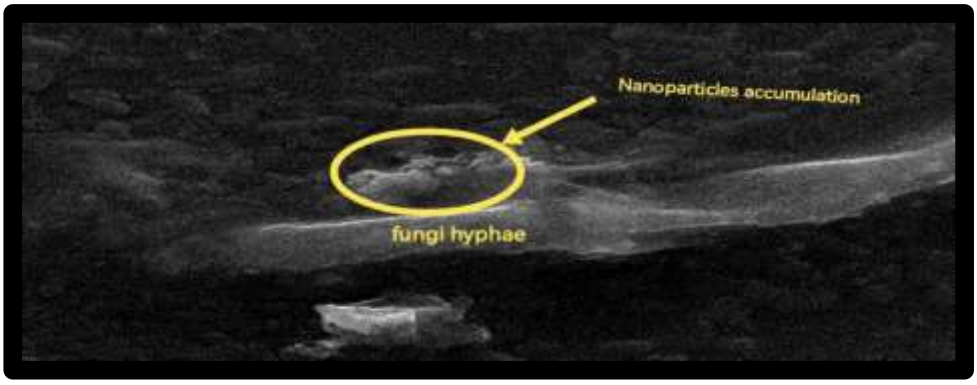

Figure 2. SEM examination image shows the distribution of gold particles on the fungal hyphae.

Measuring the sizes of gold nanoparticles accumulated in fungal mycelium

The particle diameter is calculated within certain directions in the program where the particle sizes were obtained as in Table (2). The concentration of 6 ml was the largest size of the gold nanoparticle, which reached 20.90 nm, followed by the concentration of 4 and 2 ml with a size of (14.60 and 10.50) nm, respectively .The particle size can be controlled by controlling on the concentration of the base materia., The relationship between the size of the gold particles and the concentration of the colloidal gold solution is a direct relationship, as the higher the concentration, the greater the volume, and vice versa [59].

Table 2. the sizes of gold nanoparticles accumulated in fungal mycelium

Fungal	Concentration of Au/ml	Gold nanoparticles/nm
F. oxysporum	2	10.50 4.80±
	4	14.60 7.40±
	6	20.90 10.30±

Detection of gold nanoparticles in the stem and leaves of Two varieties of mustard plant

One of the most important objectives of this research is to obtain gold nanoparticles accumulated in the vegetative parts of the plant. The results of the examination using a scanning electron microscope (SEM) showed the percentages of accumulation of gold particles in the stem and leaves of mustard as in Table (3). However , the highest percentage of gold accumulation was in the cultivar *Brassica junceae*, at a concentration of 2 ml of colloidal gold, reached 0.1% in the stem compared to the leaves, where it reached 0.2%. Tthe concentration of 4 ml recorded the same percentage in the stem and leaves, while the concentration of 6 ml recorded the opposite result of the low concentration, as the percentage of Gold nanoparticles in the stem reached 0.2%. The leaves are 0.1%, while the wild variety *Sinapis arvenis* not record any accumulation percentage in the high concentration of 6 ml, while it recorded 0.3% of gold in the stem and 0.1 in the leaves. The ability of the Indian variety to accumulate Gold nanoparticles in its parts may be due to the behavior of the variety itself in absorbing and withdrawing ions The mineral elements or the age of the plant may be related to the speed and slow absorption of the mineral [60]It was noticed that the Brassica juncea was able to accumulate heavy metal nanoparticles in the stem, and this makes it a suitable plant in environmental treatment, as this examination is considered a confirmatory result for the presence of gold nanoparticles in the shoot. Both plant varieties, and this test gives us information about the morphological composition of the gold nanoparticles [60], [61].

Table 3. The percentage of gold nanoparticles accumulated in different parts of the plants.

Varaity	Concentration Au/ml	Stem (Au%)	Leaves (Au%)
	2	0.1	0.2
Brassica junceae	4	0.1	0.1
	6	0.2	0.1
	2	0.3	0.1
Sinapis arvenis	4	0.1	0.0
	6	0.0	0.0

Measurement of gold nanoparticle sizes in stem and leaves of both mustard varieties

The results showed that there were differences between the sizes in different concentrations of the *Brassica junceae* and *Sinapis arvenis*, where the *Brassica junceae* variety out performed the *Sinapis arvenis* one in particle size at different concentrations and reached. The maximum size was 976.73 nm at low concentration in the stem, while the lowest size was 105.01 nm at concentration 4 ml. As for the leaves, the maximum size of gold nanoparticles at concentration 2 ml reached 344.33 nm, while the lowest size was at a high 6 ml concentration 39.38 nm. As for the *Sinapis arvenis* variety, it reached the maximum size of gold nanoparticles at a concentration of 2 ml has a size of 905.10 nm, followed by a concentration of 6 and 4 ml in the stem. However , the leaves have a maximum size of 1273.75 nm at a concentration of 6 ml and a minimum size of 20.10 at a low concentration of 2 ml [61], [62] as In Table (4), which explains the relationship of size with concentrations in controlling particle sizes, as [62] was able to obtain gold nanoparticles with sizes ranging from 8, 35 and 18 in the root, stem and leaves, respectively, at a concentration of 6 ml.

Table 4. Measurement of gold nanoparticle sizes in stem and leaves of both mustard varieties.

Varaity	Concentration Au/ml	Stem Gold particles Size nm	Leaves Gold particles size nm
	2	976.73 425.02±	344.33 130.20±
Brassica junceae	4	105.01 56.54±	43.44 10.36±
	6	479.08 141.86±	39.38 9.18±
	2	905.10 498.40±	20.10 8.01±
Sinapis arvenis	4	62.12 31.04±	21.23 11.21±
	6	63.80 8.72±	1273.75 1126.93±

Conclusion:

The technology of producing gold particles from the biological system of microorganisms (fungi) and plants (Two varieties of mustard plant) is a modern science that has many applications. The use of gold particle engineering in plant production on a large scale showed positive and negative effects on the plant system, where these particles enter the plant with certain mechanisms that depend on the size of the particles, and changes

occur in the physiological, chemical and biophysical system, stimulating the process of oxidation and reduction of the gold metal. Low efficiency in increasing the activity of enzymes. It also showed the ability of F.oxysporum to biologically oxidize metals through the proteins and natural enzymes formed in it. Where gold particles accumulated on the fungal hyphae were detected at a rate of 4.2% as a result of its oxidation of the three-charge gold to a pure metal at a concentration of 6 ml and a size of 20.90 nanometers. 0.1% for the Indian variety with a size of 976.73 nm and leaves by 0.2% with a size of 344.33 nm for low concentrations. As for the wild variety, the percentage of gold particles in the stem reached 0.3% and a size of 1273.75 nm for the same low concentration, which indicates that the varieties have the ability to accumulate gold in its parts and in varying proportions of the variety to another depending on the concentration used.

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