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Biosynthesis of nano- iron sulfate using fenugreek plant and evaluation of its activity against white mold disease on eggplant

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Abstract: This study was conducted to evaluate the effectiveness of regular and nano-fenugreek seeds at three concentrations and the alcoholic extract of fenugreek leaves and seeds at three concentrations (400, 800, 1200) ppm in resisting the fungus *Sclerotinia sclerrotiorum*, which causes white mold disease on eggplant , The results showed the effectiveness of nano-fenugreek seeds and the leaves and seeds of the fenugreek plant in reducing the severity of infection with the disease, with significant differences from the treatment of the pathogenic fungus at a concentration of 3% for the nano-fenugreek and a concentration of 1200 ppm for the leaves and seeds of the regular fenugreek plant. The results also showed that all treatments had a positive impact on the growth parameters of the eggplant plant, represented by length. Plant, leaf length and width, and wet and dry weight of plants compared to the Treatment of pathogenic fungi.
Keyword: eggplant, seclertinia, nanoparticles

INTRODUCTION :

Eggplant, Solanium melongena, is one of the important economic vegetable crops in Iraq and belongs to the Solanaceae family , Its cultivation is widespread in tropical and subtropical regions, and its original homeland is India, central and southern China (Khalil, 2004).Its fruits contain vitamins and nutrients such as phosphorus, iron, and calcium (Al-Rikabi and Al-Mashal, 1981). Eggplant is grown in open fields in early spring as a summer crop, and its cultivation was introduced in greenhouses in the winter, so it is grown in different seasons. In protected agriculture, it is infected with a number of diseases, and the most important disease is white mold caused by the fungus *Sclerotinia sclerrotiorum*, as the disease causes major economic losses, especially in Greenhouses when environmental conditions suitable for pathogenic fungi are available, El-Behadli and Al-Azawi recorded the disease in greenhouses on eggplant in Iraq in 1979.

Control using plant extracts of pathogens are useful and harmless methods compared to chemical control methods that cause risks to human health and environmental pollution (Nautiyal, 2001).

Due to the economic importance of the fungus *S.sclerotiorum* and its infestation of a wide range of crops, it is a real problem in the production of a number of crops in the conditions of temperate or cold and humid regions. Therefore, it has become necessary to find natural control agents alternative to the use of chemical pesticides. The study aimed to evaluate the efficiency of the alcoholic extract of seeds and leaves. The fenugreek plant and nano-fenugreek seeds in fighting the pathogen, as the fenugreek plant is one of the herbaceous plants whose seeds are used for medicinal purposes, and India is its original homeland. It belongs to the leguminous family, and fenugreek is rich in proteins, sugars, and fats and has a high nutritional value (Salem, 2006) . Fenugreek extract has been used to combat fungi such as *Fusarium oxysporum*, as the effectiveness of this extract has been proven in reducing infection with this fungus (Muhammad, 2016). Nanotechnology is defined as the technology that gives us the ability to directly control materials and devices whose dimensions are less than 100 nanometers by manufacturing them and studying their properties (Knipe et al., 2013). Park et al. (2006)

pointed out that Nanoparticles (NPS) play a major role in managing plant diseases compared to manufactured fungicides, and nanoparticles can be used with relative safety to control plant pathogens compared to the use of agricultural chemicals (Mohendra et al., 2012).

MATERIALS AND METHODS OF WORK:

1- Isolation of pathogenic fungi:

Eggplant plants that showed symptoms and signs of white mold disease caused by the fungus *Sclerotinia sclerrotiorum* were collected in one of the greenhouses in Essaouira, and the collected leaves and stems were cut into small pieces (0.5 - 1) cm long. The pieces were superficially sterilized by immersing them in a 1% sodium hypochlorite solution for 2 minutes, then washed with sterile distilled water and dried on sterile filter paper. Then they were planted in plastic Petri dishes with a diameter of 9 cm equipped with the culture medium (PDA) Potato Dextrose Agar. The dishes were incubated at 20°C and the isolates were purified after (4-5) days by transferring part of the edges of the colony's fungal growth to petri dishes with a diameter of 9 cm containing the culture medium (PDA) (Nima, 2012).

2- Preparation of alcoholic extract of fenugreek leaves and seeds:

To make the extract, fenugreek seeds, Trigonella foenum - graecum, were brought from local markets. The dried plant sample prepared for extraction was ground using a Waring blender electric grinder (Al-Azzawi, 2011). As for the fenugreek leaves, they were collected from the market, washed in the laboratory with sterile distilled water, dried in the sun for 7 days, and then ground using an electric grinder (Hamad, 2016).
Take 100 grams of powder for each sample (fenugreek leaves and seeds) and place it in a 500 ml glass beaker, then add 200 ml of ethyl alcohol at a concentration of 70%. The mouth of the beaker is closed with a stopper and shaken for 24 hours using a shaker at medium speed and at room temperature. Then the extract is filtered through Several layers of gauze were then filtered through filter paper in a Buechner funnel, then the solution was poured into petri dishes and left in the oven for 24 hours at a temperature of 45°C, after which the extract was collected in sterile bottles and preserved until use (Mohamed, 2012).

3- Preparation of the nanoring:

The experiment was conducted in the Nanotechnology Laboratory - Department of Biotechnology at the College of Science - University of Baghdad. 1 g of the alcoholic extract of the fenugreek plant was taken and dissolved in 200 ml of distilled deionized water. Place it in an ultrasonic bath for 60 minutes for the purpose of dissolving and homogenizing the mixture, as ultrasonic waves work to increase the solubility of the extract in water, homogenize the mixture, and increase the solubility of the extract in water.

Then distribute the extract dissolved with water into 10 ml tubes and place it in a centrifuge (400 revolutions for ten minutes) to obtain a filtrate and a precipitate. The filtrate was taken and for every 200 ml of it, 2 grams of Ferrous Sulphate Heptahydrate FeSo4 were added. Its color changed to a dark color (blackish green), which is evidence of the transformation into nanoparticles. The mixture was placed on the Magnetic Stirrer for an hour to increase mixing. The mixture was distributed in tubes. It was placed in a centrifuge to settle the. The sediment was taken and represents iron nanoparticles (Fe2O3NPs), collected in dishes and placed in the oven for 10 minutes at a temperature of 100°C to dry (Kiruba Daniel et al., 2013).

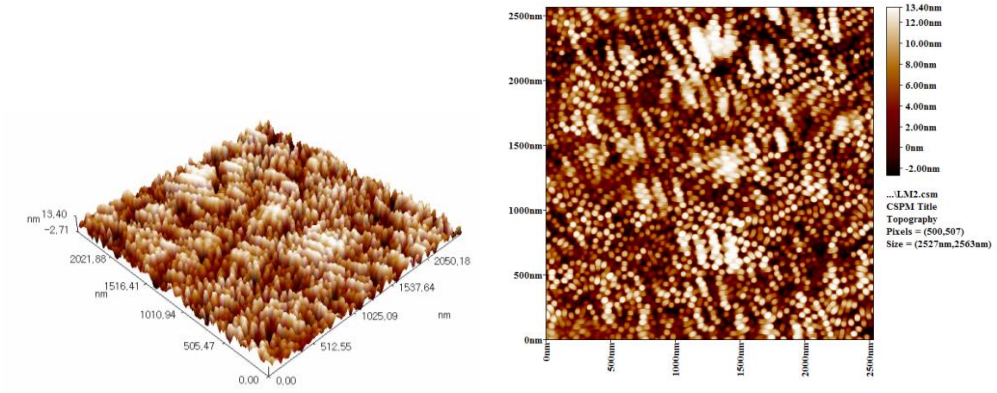
Nanomaterial screening

Atomic force microscope (AFM):

For the purpose of determining the structural characteristics in terms of size and shape of the nanomaterial formed, drops of the solution formed by mixing the alcoholic extract with iron sulphate were taken after the color changed to dark. The drops were placed on a glass slide and left to dry.

Then it was sent for examination using an atomic force microscope (AFM) in the laboratories of the Chemistry Department - College of Science - University of Baghdad. As atomic force microscope images showed that the iron nanoparticles formed had different shapes and sizes, Figure (1) their average size is 56.55 nanometers (Pailleret 2007,).

Avg. Diameter: 56.55 nm	**<=10% Diameter: 45.00 nm**
<=50% Diameter: 55.00 nm	**<=90% Diameter: 65.00 nm**



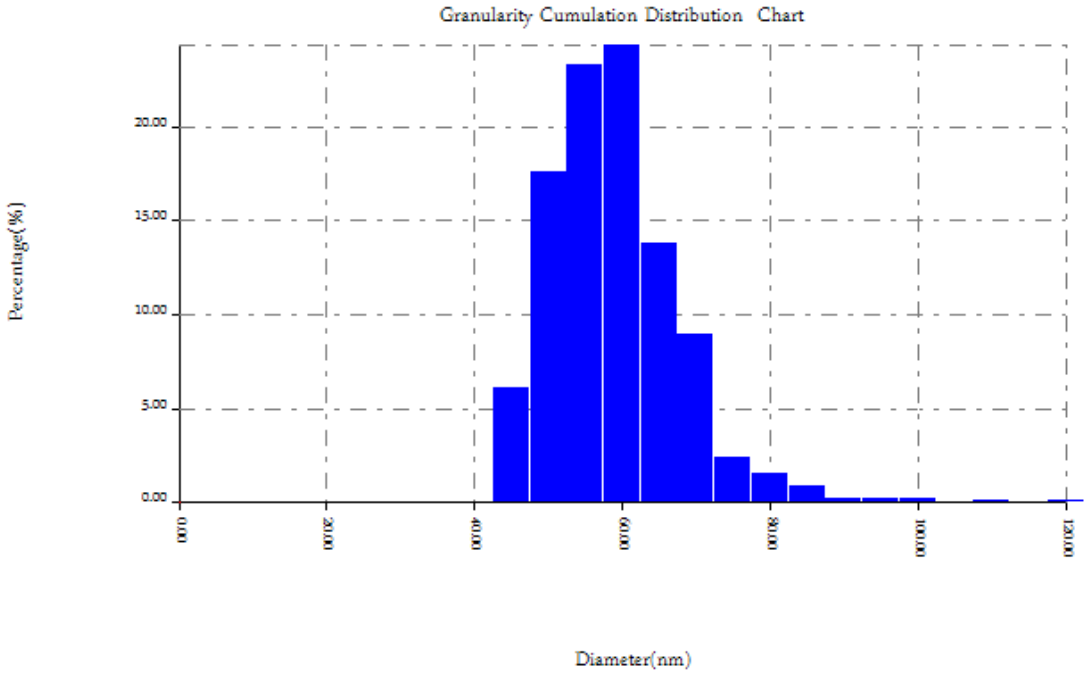


Figure (1) shows the size of the nanomaterial

4- Laboratory experiments:

The efficiency of the control agents against the fungus *Sclerotinia sclerrotiorum* was tested using the poisoned media method. Three concentrations (400, 800, 1200) ppm were made for each control agent (leaves and seeds) and three replicates for each concentration. The nanorings were also added to the PDA medium in glass flasks before sterilization at concentrations (400). , 800, 1200 ppm, 3 replicates for each concentration. Then the best concentration is chosen for use in the greenhouse experiments. I used 100 ml conical glass flasks containing 60 ml of the ready-made PDA medium after sterilizing them with an autoclave. A stock solution is made from the extract of the seeds and leaves of the fenugreek plant for the purpose of adding them to the PDA medium according to the chosen concentrations. Shaking it in a regular circular motion to ensure homogeneity of the contents, then pouring it into petri dishes with a diameter of 9 cm, then inoculating each dish with a disk with a diameter of 0.5 mm taken from the edge of a colony of the fungus *S.sclerotiorum*, which is (5-6) days old. The disk is placed in the center of each poisoned dish in addition to the PDA dishes. The non-poisoned (comparison) mushrooms were added to them only and incubated at a temperature of 20°C. The readings were taken three days after inoculation, where the fungal growth rate was taken and the percentage of inhibition was calculated for each treatment according to the following equation:

Percentage of inhibition = (average diameter of comparison - average diameter of treatment) / average diameter of comparison × 100

5- Testing the efficiency of the control agents used in the study against the pathogenic fungus S.sclerotiorum in greenhouse conditions:

The experiment was conducted in the greenhouse conditions of the Department of Plant Protection / College of Agricultural Engineering Sciences / University of Baghdad. A mixture

of mixed soil and peat moss was used in a ratio of 1:2 after sterilizing it in the incubator twice. Eggplant seedlings were planted at one month old, with three replicates for each treatment and two plants for each replicate. Then the experiment was conducted Necessary agricultural operations, after industrial infection of plants with pathogenic fungi grown on PDA culture medium according to the method (Petzoldt & Dickson, 1996), by making a wound 1 cm long and 1 mm deep on the main stem of each plant, with a portion of the fungal isolate placed on the wound taken from the edge Isolate a fungal colony at 5-6 days old using a cork auger with a diameter of 0.5 mm, then cover the pots with polyethylene bags to maintain humidity. Then, readings were taken weekly, which included calculating the severity of the infection, for a full month after the inoculation procedure, and the severity of the infection was recorded according to the scale established by (Dixon & Doodson, 1971): 0: no infection - 1: the stem was surrounded by fungal rot by an amount of less than 1/2. 2: Surrounding the stem with fungal rot by 1/2 to less than complete encircling. 3: Completely surrounding the stem with rot. 4: Plant death.

The severity of the injury was calculated according to the McKinny equation (Mckinny, 1923) as follows:

Infestation severity % = (number of plants of grade 0 ×0 + ... + number of plants of grade 4 ×4) / total number of plants examined × 4) × 100. The fresh and dry weight and length of the plants were also calculated after the plants were uprooted and their root system was washed with running water to remove any dust attached to it. They were placed in paper bags and dried in an electric oven for 48 hours.

RESULTS AND DISCUSSION :

Laboratory testing the efficiency of resistance agents against the fungus *S.sclerotiorum:*

The results of testing the efficiency of the alcoholic extract of fenugreek (seeds, leaves) and nano-fenugreek (Table 1) showed that the nano-fenugreek treatment at concentrations (400, 800, 1200) ppm was superior to the rest of the treatments, as the inhibition rate reached (87.44, 87.67, 100)%, respectively. Followed by treatment with the alcoholic extract of fenugreek seeds at concentrations of 400, 800, and 1200 parts per million, where the inhibition rate reached 85.56, 86.33, and 100%, respectively, while the inhibition rate of the alcoholic extract of fenugreek leaves reached 22.65, 67.47, and 83.67%, respectively. These are the results. It is consistent with what was found by Hamad et al. (2016) that the seeds contain a higher percentage of alkaloids and soapy substances than the leaves, which therefore affected the growth of hyphae of the fungus *S.sclerotiorum*.

Table 1: The effect of alcoholic extract of both leaves and seeds of fenugreek and nano-fenugreek on the growth of mushrooms grown on the nutrient medium PDA:

treatment	PPM concentrations	Colony growth rate (cm)	Inhibition rate %
control	0	9.00	0.00
Alcoholic extract of leaves	400	3.13	65.22
	800	2.28	74.67
	1200	1.47	83.67
Alcoholic extract of seeds	400	1.30	85.56
	800	1.23	86.33
	1200	0.00	100
Nano fenugreek	400	1.13	87.44

	800	1.11	87.67
	1200	0.00	100

Testing the effectiveness of the control agents used in the study against the pathogenic fungus *Sclerotinia sclerrotiorum* in greenhouse conditions:
The results (Table 2) indicated the efficiency of nano-fenugreek in reducing the severity of infection with the pathogenic fungus at a concentration of 3%, as the severity of the infection reached 25.21%, followed by testing the alcoholic extract of fenugreek leaves and seeds at a concentration of 1200 PPM, which reduced the severity of the infection as it reached (41.62, 30.72). % respectively compared to the control treatment (without addition), as the severity of the infection reached 93.33%. These results agreed with what was stated by Al-Mughrabi and others (2010) that the role of saponins in resisting pathogens is through their action as a barrier that prevents pathogens from attacking the plant. They also work to increase plant health, meaning that saponins work like antibiotics, thus protecting the plant from pathogens. It stimulates the plant to produce compounds that act as lipid-destroying substances in mycelium cells (Popdoulou et al., 1999). Since the cell wall acts as a barrier between the external environment and the internal components of the fungus, the effect of saponins is to dissolve the fats present in the fungal cell wall and make the plasma membrane completely permeable instead of it being optionally permeable (Al -Mughrabi et al., 2010). As for the efficiency of nanoparticles, it is due to increasing the area. Surface to volume and physical, chemical and biological properties increase compared to normal volumes of materials (Dos Santos et al., 2014).

The effect of the treatments on fresh and dry plant weight was statistically clear, as all treatments showed a significant increase in fresh and dry weight compared to the control treatment (pathogenic fungus only). The nanofenugreek treatment achieved the highest average fresh and dry weight, reaching (5.11, 0.89) grams/plant. The average fresh and dry weight of the plant was followed by treatment with alcoholic extract of seeds, which amounted to (3.79, 0.77) g/plant, respectively. This treatment did not differ significantly from treatment with alcoholic extract of fenugreek leaves, which amounted to (3.71, 0.58) g/plant, respectively.

The treatments showed an effect on leaf length and width, as all treatments had superior leaf length and width compared to the control treatment. The alcoholic extract of fenugreek seeds achieved the highest rate of leaf length and width, reaching (4.38 and 66.09) cm/leaf, respectively. These treatments did not differ significantly from the treatments. The alcoholic extract of fenugreek leaves reached (5.88, 3.81) cm/leaf, respectively. As for the nano-fenugreek seed treatment, the length and width of the leaf reached (7.59, 5.89) cm/leaf, respectively
As for plant lengths, the two treatments of fenugreek nanoseeds and the alcoholic extract of fenugreek seeds were distinguished compared to the control treatment, with clear, statistically significant differences, as the plant length for the two treatments reached (33.89, 32.53) cm/plant, while the comparison treatment reached 30.3 cm/plant.
The reason for the increase in plant growth is due to treatment with alcoholic extracts of the seeds and leaves of the fenugreek plant, which contains saponin, which has a role in inducing systemic resistance and resistance to pathogens (Al-Mughrabi et al., 2010). As for the reason for the decrease in fresh and dry weight and plant height in the treatment of the pathogenic fungus than in the treatment of In comparison, only sterilized soil was used. This may be due to the fact that the infection reduces the germination rate and also leads to increased respiration of the diseased plants, which reflects the weakness and stunting of the plant. The infection also leads to an obstruction of the transfer of water and nutrients (Garrett, 1970).

This result was consistent with Hussein (2017), who confirmed the effectiveness of nanoparticles of nano-magnesium oxide in increasing plant height, root mass, and fresh and dry weight of watermelon plants. Al-Jawthari (2017) stated that using nano-iron and nano-zinc on brocade plants recorded the highest plant height and the highest fresh weight. Dry and number of leaves compared to the control treatment.

Table. 2 Comparison of different concentrations of alcoholic extract of fenugreek leaves and seeds in resistance to the fungus *Sclerotinia sclerrotiorum* in greenhouse conditions.

treatment	Injury severity %	Plant length cm	Leaf length cm	View the plant leaf cm	Fresh weight /g	Dry weight /g
Control without fungus	0.00	32.39	7.07	4.09	3.26	0.74
Control with the addition of pathogenic fungi only	91.33	30.3	5.51	2.67	2.43	0.55
Alcoholic extract of leaves at a concentration of 1200ppm + pathogenic fungus	41.62	32.14	5.88	3.81	3.71	0.58
Alcoholic extract of seeds at a concentration of 1200ppm + pathogenic fungi	30.72	32.53	6.09	4.38	3.79	0.77
Nano at a fenugreek concentration of 1200 ppm	25.21	33.89	7.59	5.89	5.11	0.89
L.S.D. 0.05	8.95	2.22	1.09	0.33	0.70	1.19

CONCLUSIONS AND RECOMMENDATIONS:

Based on the results of the current study, we can conclude that nano-fenugreek, the alcoholic extract of the seeds and leaves of the fenugreek plant, is efficient in inhibiting the mycelial growth of the fungus *Sclerotinia sclerrotiorum*, in addition to the possibility of studying the time period required for the effectiveness of the alcoholic extract of the leaves and seeds of the nano-fenugreek plant on the plants sprayed with it, and conducting subsequent studies on it. The possibility of separating the active ingredients present in fenugreek plant extracts in order to manufacture a biopesticide from them after testing their effects on the environment and public health.

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