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FUSARIUM WILT ON SUMAC PLANT : PATHOGENICITY AND MANAGEMENT POTENTIALS USING SOME NANO-MATERIALS

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Abstract

This is the first study in Iraq on *Fusarium* wilt disease and the fungi associated with the sumac plants, the pathogenicity tests showed that the most frequent fungus *Fusarium oxysporum* caused the plant's symptoms of upward yellowing, wilted leaves followed pale appearance, then dry leaves and stalk one month post inoculation. Symptoms also appeared on one side of the plant and brown discoloration was observed in the wood vessels in the longitudinal section of the stem and roots of the affected plant.

Nanoparticles have also been tested *in vitro* for their inhibitory effect against *F. oxysporium*. Results showed that inhibition rate increased as the nanomaterial concentration increases, the highest inhibition rate was always recorded at the highest concentration (400 ppm). Nano-silica at 400 ppm and the Nano-mixture (silica, silver and magnesium) at concentrations of 200 and 400 ppm were significantly superior to all the other treatments with inhibition rates of 87.22%, 77.72% and 84.54%, respectively.

In case of *Fusarium* wilt management using Nano-particles and a fungicide in the greenhouse, the highest disease incidence (92.5%) was recorded in the infected untreated plants followed by treatments Nano-MgO and Nano-silica while the least incidence was recorded in the Beltanol treatment which did not differ from Nano-silver and the Nano-mixture. The highest disease severity was recorded in the infected untreated plants 0.46 while all the other treatments significantly reduced disease severity that ranging from 0.23 to 0.12. As for horticultural traits, the highest plant height was recorded in the treatment of the Nano-mixture of non-inoculated plants. The number of branches in all treatments was significantly higher than the untreated infected plants. The highest number of leaves was 26.50 and 25.25 in the treatment of tri Nano-mixture and Nano-silver treatments for non-inoculated plants, respectively. The highest percentage of total chlorophyll content (86.05) was recorded in Nano-silica treatment in the presence of the pathogen.

Key words: *Fusarium*, sumac, Nano-silver, Nano-silica, Nano magnesium oxide.

Introduction

The sumac plant is named after English Sumac or Summaq and its Latin name Rhus means red, a reference to the red color of the leaves, flowers and fruits of the sumac at maturity. It contains 91 species (The plant list, 2010) belonging to the family Anacaridiaceae (Maadadi, 2006). Sumac is a plant adapted to different environments, succeeds in the mountainous areas and can be planted as fence and windbreaks around orchards. It also grows on the edges of waterways, hills and marine rocks.

Sumac is a summer spring plant that usually grows from February to November. It is grown as an economic plant in the environment and favorable conditions. Its trees grow in rough and semi-arid areas and withstand harsh environmental conditions. It is cultivated for its economic return and to prevent soil erosion. Sumac is prevalent in Iraq in the northern, northeast and western regions of the country, as well as in mountainous environments, especially in Amadiyah, Rawanduz, Sulaymaniyah and JabalSinjar (Al-Ma'adi, 2006).Sumac has important medical benefits. It is used in the treatment of wounds and ulcers, gastrointestinal diseases, preserves the health of the liver and increases the secretion of the biliary gland, maintains cardiovascular health. Sumac is used in the manufacture of many medicines, oils and aromatic substances, appetizing and flavoring for food. Contains

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vitamin C which is a powerful antioxidant that eliminates the free radicals of cells. Helps lose body weight. Sumac is involved in preparing salads and many cooked or preserved foods (Al-Seyouf and Al-Rashida, 2007, Ibrahim *et al*., 2014; Abu-Reidah *et al*., 2014).

Like other plants, sumac is infected with many fungal, bacterial, nematode and viral diseases. Fusarium wilt is one of the most common and common fungal diseases that affect plants in general, especially in climate-friendly countries. Fusarium wilt in Iraq is generally found on many plants and in Nineveh province in particular. The disease is caused by the Fusarium fungus which is able to keep in the soil for a long time. The fungus affects seedlings in the nursery and leads to its death, as it affects large plants causing a clear shortage of the crop.

One of the safest and harmless methods on humans and the environment as an alternative to the harmful chemical control is biological control using organisms that have been successful against soil borne plant pathogens (Bahattacharyya and Jha, 2012). But generally, biological control is relatively time required to be effective. Nanotechnology has recently emerged, with agriculture being second most used in the list nanotechnology implementation, which can play an important role in global food production and food safety (Jabouri, 2006).

The nanoparticles of some substances were used to control plant diseases. The most widely used nanoparticles were carbon, silver and silica. Silver nanoparticles were used to control powdery mildew on roses caused by *Sphaerotheca pannosa* var. *rosa* is widespread in greenhouses, which reduced the disease by 95% after two days of spraying (Mehta and Award, 2011). It was noted that the use of silver nanoparticles inhibited the growth of cystic fungi such as *Rhizoctonia solani* and *Sclerotinia sclerotium* and also prevented the germination of the stone bodies of these fungi. Kasprowicz *et al*., 2010, explained that silver nanoparticles inhibit the growth of stone bodies formed by certain fungi as well as inhibiting *Fusarium* spp. Aguilar Mendez *et al*., 2011, also noted that silver nanoparticles inhibited the growth of the mycelium of *Colletotricum gloeosporiodes*, which causes anthracnose disease in fruits. Yet, no any study or a report on the diseases that affect sumac plants in Iraq, especially fungal diseases, including Fusarium wilt disease on the sumac. Therefore, this study aimed to identify the fungi associated with the sumac plant with a focus on pathogenicity of the most frequent fungi and to *in vitro* evaluate effect of some Nano-materials at different concentration and combinations on these fungi compared fungicide Beltanol.

Materials and Methods

Isolation and diagnosis

Samples were taken from the stem and branches of the infected sumac plants (previously brought from the infected fields) and washed under running water for two hours to remove the suspended dust. They were then cut by a sterile scalpel into 0.5 cm long pieces and superficially sterilized with 1% sodium hypochlorite solution (NaOCl) for two minutes, dried between two filter papers and then cultured in Petri dishes containing Potato Dextrose Agar (PDA) at 5 pieces per dish. The dishes were incubated at 25 ± 2°C for five days and the fungal growth was purified for identification and used in subsequent experiments. The frequency of each isolated fungus was calculated (REF).

Inoculum preparation

Fusarium oxysporum inoculum was prepared using eight-day-old pure colony to inoculate local millet seeds *Panicum miliaceum* L. at rate of 5 tablets of 5 mm diameter to each 250 g seeds (Dewan, 1989).

Pathogenicity tests

The pathogenicity test of the most frequent fungi was performed on sumac seedlings obtained from Aqra nursery already planted in 3 Kg plastic bags containing 1% formalin sterile soil (Mustaffe and Chattopadhyay, 1981). Millet seeds contaminated with pure fungi were used at rate of 10g seeds added to the soil of each sumac seedling. Five seedlings were used for each test and five non-inoculated seedlings served as control. Plants were daily observed to record infection symptoms where the causal fungi were isolated from infected plants was assured to confirm Koch's pathogenesis.

Effect of Nano-materials on *in vitro* fungal growth of *F.oxysporium*

The effect of three Nano-materials (magnesium oxide, silver and silica) individually or combined together (mixture of the three Nano-materials) at two concentrations (200 and 400 ppm) was tested on fungal radial growth of *F. oxysporium* in Petri dishes containing PDA nutrient medium. The PDA was mixed with Nano-materials at each concentration and the mixture was autoclaved. Chlorophenicol was added to the sterilized medium, mixed well then the medium was distributed by 20 ml in Petri dishes. The center of each dish was inoculated with a 0.5 cm diameter disc grown from pure pathogenic fungus on the PDA medium and incubated at 25 ± 2°C. The experiment was Completely Randomized Design CRD with three replicates, three dishes per treatment and per concentration, inoculated untreated (free of any Nano-

material) served as control. (Note: in the initial experiment, lower concentration were used 50 ppm, 100 ppm and 150 ppm of all nanomaterial test).

Five day post inoculation, data were recorded and diameter of each developing colony in each dish was calculated. The inhibition rate of fungal growth of different treatments over the control treatment was calculated according to the Abbot (1925) equation following Shaban and Al-Malah (1993) using the following formula:

$$\%\text{inhibition} = \frac{\text{Control colony diameter - treatment diameter}}{\text{Control colony diameter}} \times 100$$

Nano-materials and Chemical Control of *Fusarium* Wilt Disease in Plastic House

Nano-materials of magnesium oxide, silver and silica in comparison with fungicide Beltanol were evaluated for their effectiveness to control *Fusarium* wilt disease on the sumac under plastic house conditions. Sumac seeds were planted in 3 Kg plastic bags containing sterile soil incorporated with 10 g *F. oxysporium* infected millet seeds. The experiment was CRD with 12 treatments and five replicates (plants). The treatments included three Nano-materials and Nano-mixture at concentration of 400 ppm with or without pathogen, negative untreated non-inoculated control, positive inoculated untreated control in addition to treatment of fungicide Beltanol (2 ml/liter) added to plants at rate of 20ml per panting bag of inoculated and non-inoculated.

Data were recorded two months post planting and disease incidence was estimated based on the symptoms shown on the vegetative plant parts. The symptoms included appearing of yellowing and dryness on leaves, leaf death and death of branches. A five-degree index (0-4) estimated by the naked eye was used to assess infection severity where 0= healthy plant, 1 = 1-50% shoot wilt, 2 = 51-100% shoot wilt, 3 = totally wilted shoot with green stem and 4 = dead plant.

Data of all the experiments were analyzed and subjected for analysis of variance ANOVA using Gen Stat 2nd Edition computing program. Means were compared according Duncan's multiple range tests at $P \leq 0.05$.

Results and Discussion

Isolation and identification

The results of laboratory isolation showed the presence of several fungal pathogens on different plant parts roots, crown area, stems and branches. *Fusarium*

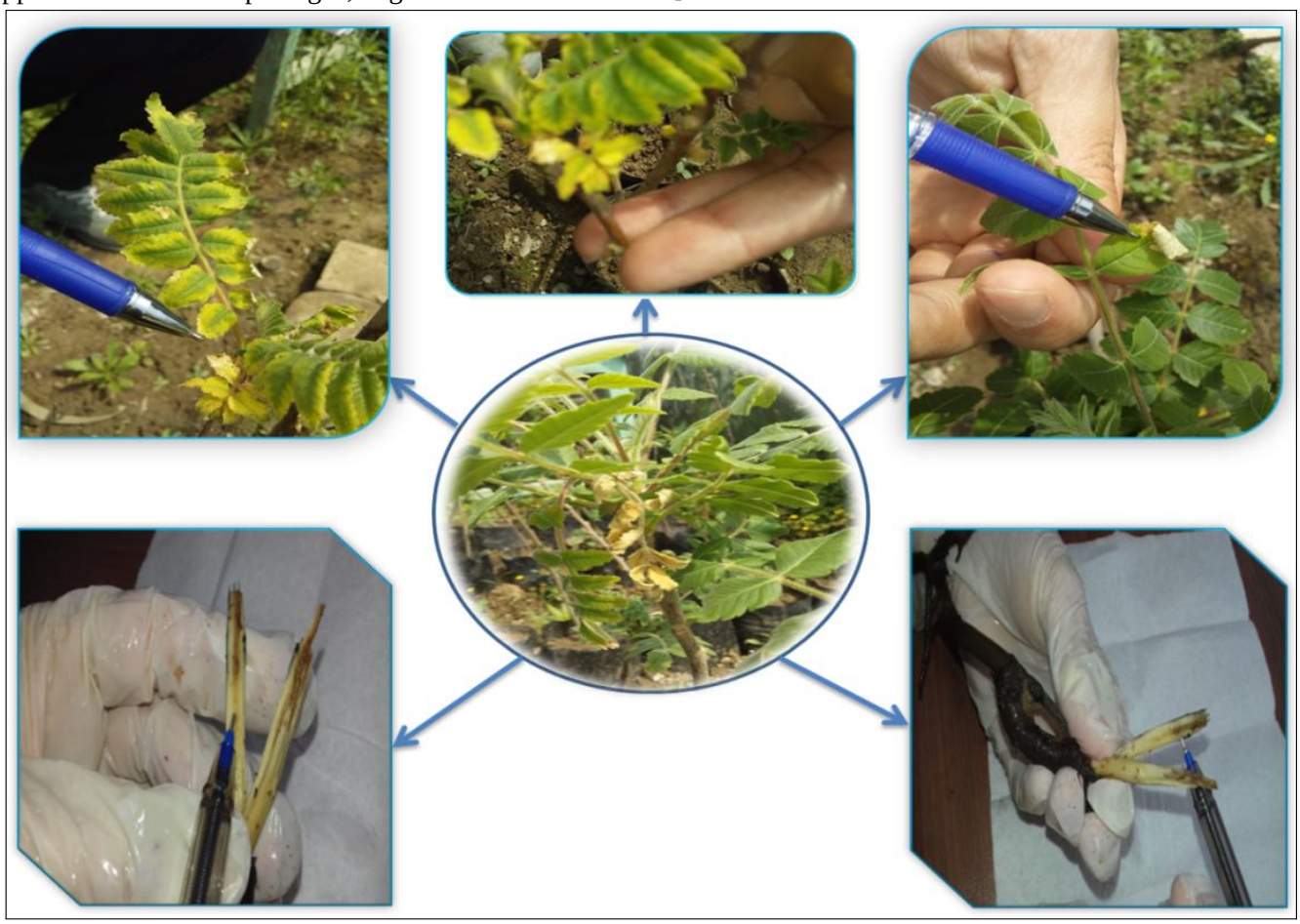

Fig. 1: Disease symptoms caused by *F. oxysporum* f. sp *Rhus* on sumac plants.

Table 1: Effect of Nano-materials on *in vitro* fungal growth of *F. oxysporium*.

Treatment		% inhibition	Colony diameter (cm)
Control		0.00 e	8.50 a
Nano-silver	200 ppm	65.14 cd	2.67 bc
	400 ppm	67.22 cd	267bc
Nano-Magnesium Oxide	200 ppm	59.58 d	3.17 b
	400 ppm	65.00 cd	2.67 bc
Nano-Silica	200 ppm	78.03 bc	2.00 cd
	400 ppm	87.22 a	1.00 e
Nano-mixture	200 ppm	77.72 ab	1.67 de
	400 ppm	84.54 a	1.17 c

oxysporum had the highest recurrence rate and *F. oxysporum* was confirmed based on the morphological characteristics of the pure single fungal colony cultured on PDA. The colony showed white cotton appearance with slight pink color changing to dark red in more aged colonies. The colony was 8.5 cm in diameter after eight days of incubation at 25 ± 2°C. Microscopic examination showed the appearance of the three spore types produced by the fungus which were identical to the morphological descriptions of *Fusarium oxysporum* f. sp. *Rhus* according to Nelson *et al*., (1983) and Leslie and Summerell, (2006).

Pathogenicity test

The pathogenicity of *F. oxysporum* f. sp *Rhus* isolated from sumac plants was tested. Pathogenic symptoms appeared in the greenhouse at the beginning of April, 40 days after infection. The severity of the disease increased in May with the emergence of infection on all inoculated sumac plants. Symptoms included upward yellowing and wilted leaves followed pale appearance, then dry leaves and stalk one month post inoculation. Symptoms also appeared on one side of the plant and brown discoloration was observed in the wood vessels in the longitudinal section of the stem and roots of the infected plant parts (Fig. 1). This confirms the ability of the fungus to cause infection, which was isolated from the affected area. After the fungus enters the wooden vessels of the delicate plant, it begins to secrete pectin-decomposing enzymes (*e.g*., pectin enzymes, methylastases and polygalacturonase), which break down the insoluble pectin compounds into pectic acids that spread through the wall of the xylem vessels, forming colloidal lumps that block these vessels. Generally, the brown coloration of the vessels is resulting from the release of phenolic substances, which are rapidly decomposed by phenoloxidase enzyme activity into brown plant melanin absorbed by the vessels walls, causing the distinguished brown color of the disease. The accumulation of ethylene in the obstruction area is one of the causes of leaf yellowing as early disease symptom. The production of fusaric acid or the toxin lycomarasmin by the fungus is likely to lead to permanent wilting (aerosols and mustapha, 2011).

Effect of Nano-materials on *in vitro* fungal growth of *F.oxysporium*

The results of table 1, indicate that increasing Nano-material concentration resulted in increasing the inhibition of fungal growth in all treatments under study. Concentration of 400 ppm resulted in a noticeable increase in the inhibition ratios. The results (Table 2) also showed that the Nano-silica at 400 concentration and the mixture at 200 and 400 ppm significantly exceeded the other treatments causing inhibition rates of 87.22%, 77.72%

Table 2: Effect of different treatments on *Fusarium* wilt disease and some growth indicators of sumac plants under greenhouse conditions**.**

Treatments	Percent infection	Infection severity	Plant height	No. of branches/plant	No. of leaf/plant	Total chlorophyll
Non-inoculated control	0.00 e	0.00 d	51.10 ab	3.25 a	18.00 bc	48.38 a
Inoculated untreated	92.50 a	0.46 a	37.38 de	2.50 b	10.50 d	25.75 d
Infected +Nano MgO	58.75 b	0.12 c	46.75 bc	3.50 a	18.75 ba	42.46 abc
Infected +Nano Si	56.25 b	0.23 b	50.25 b	3.25 a	19.25 bc	45.00ab
Infected +Nano Ag	38.50 c	0.13 c	39.75 de	3.50 a	20.50 bc	37.75 d
Infected +Nano mix(MgO/Si/Ag)	38.25 c	0.06 cd	51.00 ab	3.53 a	20.00 bc	49.38 a
Nano MgO only	0.00 d	0.00	52.00 ab	3.51 a	23.75 ab	48.4 a
Nano Si only	0.00 d	0.00	54.00 a	3.25 a	18.75 bc	47.8 a
Nano Ag only	0.00 d	0.00	52.00 ab	3.22 a	25.25 a	47.50 a
Mix(MgO/Si/Ag) only	0.00 d	0.00	56.75 a	3.55 a	26.50 a	49.30 a
Infected+Beltanol	38.10 c	0.13 c	45.00 bc	3.25 a	17.00 bc	40.63 bc
Beltanol only	0.00 e	0.00 d	50.00 ab	3.25 a	18.00 bc	47.85 a

Values are means of five replicates. Means that have same letter (s) within a column are not significantly different ($P \leq 0.05$) according Duncan's multiple range test.

and 84.54%, respectively, followed by inhibition of 78.03% from Nano-silicates at 200 ppm which did not differ from the Nano-mixture treatment with the same concentration. The other treatments did not differ among each other regardless the concentration resulting in inhibition rates ranging from 59.58% to 67.22% compared to the control treatment. The reason for this inhibition is mostly due to the mechanism of action of Nano-materials. Silica and magnesium oxide nanoparticles disrupt the vital functions of the fungus as well as inhibit the growth of fungus conidia and conidiophore.

It also believed that Nano-particles reduce the acidity of the medium by dissolving water molecules and increasing the concentration of free hydrogen ion and thus reduce the acidity of the medium to become unsuitable for fungal growth (Ram and Prasad, 2014). Silver nanoparticles interrupt plant transporting systems, including ion fluxes. This dysfunction can cause rapid accumulation of silver ions, disrupting cellular processes such as metabolism and respiration. Silver ions are also known to produce reactive oxygen species when these ions interact with oxygen. Reactive oxygen species are harmful to cells, causing damage to proteins, lipids and nucleic acids. It also affects the proteins and enzymes necessary in ATP production (REF).

Nano-materials and chemical fungicide to Control *Fusarium* Wilt Disease in Plastic House

As shown in table 2, the highest incidence was recorded in untreated plants contaminated with the pathogenic fungus at 92.5%, followed by treatment of Nano-magnesium oxide and Nano-silica which reduced the incidence to 58.75% and 56.25%, respectively, with significant difference from the lowest disease incidence resulted from Nano-silver and Nano-mixture and the fungicide Beltanol which was reduced to 38.5%, 38.25% and 38.1%, respectively. The highest disease severity was 0.46 in the control treatment of the untreated infected plants with significant difference from the other treatments followed by the treatment of Nano-silica (0.23) which differed significantly from the lowest severity of the infection recorded in Nano-silver (0.13), Nano-magnesium oxide (0.12) and Beltanol (0.13) which did not differ among each other. As for horticultural traits, the treatment of nanoparticle mixture without pathogen resulted in the highest plant height (56.75 cm) followed by nanoparticle silica in non-inoculated plants (54 cm) and treatment of magnesium oxide and nanoparticles mixture treatment in the presence and absence of pathogen with plant height values of 52, 52, 51, 51 and 50 cm which did not differ among each other, respectively. The number of branches was significantly reduced to 2.50 in the treatment of untreated infected control plants, while the highest number of leaves was in the treatment of Nano-mixture and Nano-silver of the uninfected plants, 26.50 and 25.25, respectively. It is clear from the same table 2, that the highest chlorophyll content was recorded in uninfected plants, which did not differ from the case of uninfected plants treated with chemical fungicide or Nano-mixture.

Nano-materials treatments improved plant growth traits in general. Beside the formerly mentioned reasons, this can be attributed to the role of these substances in increasing plant content of sugars and total soluble solids as well as increasing the total phenol content and polyphenol oxidase activity related to plant defenses (El-Argawy *et al*., 2017). All these activities were reflected in general plant health due to antagonistic and antibiotic effects against the pathogenic fungus, thus led to an improvement in vegetative growth traits.

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