



## Identifying The Cause of Dracaena Plant Wilt Disease in Iraq and Testing Some Nanomaterials Against It

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### Abstract

The experiments were conducted at the College of Agricultural Engineering Sciences, Plant Protection Department, University of Baghdad. Morphological identification of the pathogen agent revealed it to be the *Fusarium oxysporum* fungus. Identification was also affirmed molecularly using the general primers ITS1/ITS4 which yielded a 500 bp band of molecular weight when amplifying the internal transcribed spacer (ITS) region in the most pathogenic isolate using polymerase chain reaction (PCR). A hit of 100% identity was achieved with the worldwide isolates in the NCBI GenBank. Sequencing data obtained from the fungal isolate were submitted to the gene bank with accession number PP273503. These data revealed that the isolates ranged from highly to weakly pathogenic. Both general and nanoparticle iron and copper oxides could prevent the growth of the pathogenic fungus. The treatment with nano iron oxide recorded the highest inhibition rate of 100% at 3% concentration showing no fungal growth compared to S. copper oxide's at 77.0% for the same concentration. The findings also confirmed that both materials offered 100% control of fungal development on the Dracaena leaves in a laboratory setting at 0.0 cm<sup>2</sup> compared to 70.91 mm<sup>2</sup> and 75.88 mm<sup>2</sup> in the ordinator iron and copper oxide treatments, respectively. The materials showed the ability to control the fungus when applied with iron

and copper oxide at 69.88% and 67.77% in the Dracaena plants, 0% in the treatment containing only the pathogenic fungus, and 100% with the nano iron and copper oxide treatment.

**Keywords:** Fusarium sp., Nanoxides, Leaf spot, Iron oxide, Copper oxide.

## تحديد مسبب مرض ذبول نبات الدراسينيا في العراق واختبار بعض المواد النانوية في مكافحته

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### الخلاصة

اجريت الدراسة في مختبرات قسم وقاية النبات/ كلية علوم الهندسة الزراعية/ جامعة بغداد. اظهرت نتائج تشخيص المسبب المرضي مظهرها ان الفطر هو *Fusarium oxysporum* واكد التشخيص جزيئياً باستخدام البادئ العام ITS1/ITS4 اذا أعطت باندات ذات وزن جزيئي 500 bp عند مضاعفة منطقة النسخ الداخلي (ITS) للعزلة الاكثر امراضية بواسطة تقانة PCR. سجلت نسبة تطابق 100% مع العزلات العالمية الموجودة في بنك الجينات العالمية NCBI اودعت التتابعات النيوكليوتيدية للعزلة الفطرية في بنك الجينات (NCBI) تحت رقم انضمام PP273503. بينت النتائج ان المقدرة الامراضية تراوحت بين شديدة الامراضية الى ضعيفة الامراضية. اظهرت فعالية اوكسيد الحديد واوكسيد النحاس بالأحجام العادية والنانوية في تثبيط نمو الفطر الممرض، اذ حققت معاملة اوكسيد الحديد النانوي اعلى معدل تثبيط للفطر بلغ 100% عند التركيز 3% اذ منع نمو الفطر نهائياً مقارنة مع اوكسيد النحاس العادي عند نفس التركيز اذ بلغت نسبة التثبيط 77.0% كما اوضحت النتائج فعالية جميع المواد المختبرة في منع نمو الفطر نهائياً على اوراق الدراسينيا مختبرياً اذ بلغت المساحة المشغولة بالفطر 0.0 سم<sup>2</sup> عند معامليتي اوكسيد الحديد والنحاس النانوية في حين سجلت 70.91 و75.88 ملم<sup>2</sup> في معامليتي اوكسيد الحديد واوكسيد النحاس. كما اظهرت المواد المستخدمة كفاءة للمكافحة عند استخدام اوكسيد الحديد والنحاس اذ بلغت 69.88 و67.77% على نبات الدراسينيا في حين سجلت 0% لمعاملة الفطر الممرض لوحده و100% في معامليتي اوكسيد الحديد والنحاس النانوية.

**كلمات مفتاحية:** Fusarium sp.، الاكاسيد النانوية، تبقع الاوراق، اوكسيد الحديد، اوكسيد النحاس.

## Introduction

The Dracaena plant (*Dracaena marginata*), among the most important ornamental plants, belongs to the family Asparagaceae (10), which includes many species, reaching up to 40 types. It is a tropical plant native to Madagascar and is abundant on nearby islands in the Indian Ocean. It is characterized as an indoor plant with evergreen leaves and irregular, thick stems that require low light. The plant is known by several names (3). Its significance lies in being an ornamental plant widely valued for its beauty, availability, and affordability (18). Additionally, it has medicinal uses, such as in treating malaria, poisoning, and diarrhea, and in reducing fever and blood pressure, various species of this plant are susceptible to specific fungal pathogens with (6) reporting that it is affected by 15 fungal diseases. Among the fungi causing infections are *Colletotrichum* and *Fusarium*, which lead to symptoms such as leaf spots and stem rot. The plant is also susceptible to *Aspergillus niger* and *Alternaria alternata* (12 and 22). (1 and 8) reported the Dracaena plant being infected by *Aspergillus niger* and *Colletotrichum dracaenophilum*, respectively. Various methods have been used to control these diseases, with chemical pesticides being among the most common and fast-acting.

However, improper pesticide use can lead to the development of resistant strains and environmental pollution (5 and 26), leading researchers to seek safer and more affordable alternatives, such as essential chemical compounds and trace mineral nutrients, which are crucial for plant growth and controlling pathogens (24). One of the materials used is iron, which plays a key role in DNA synthesis, respiration, photosynthesis, and electron transport, thereby contributing to plant growth and development, enhancing their ability to withstand abiotic stresses, and increasing resistance to fungal pathogens. Iron deficiency negatively impacts the quality and productivity of plants (21 and 32). Additionally, copper has a crucial role as an enzyme cofactor in various biological processes within the plant, such as redox reactions. It is involved in the oxidation of ascorbic acid, which is responsible for vegetative, fruit, and root growth. Copper is also a component of photosynthesis and plays a role in chlorophyll synthesis (27). It enhances plant resistance to numerous diseases by acting as an enzymatic cofactor in lignin formation.

The effectiveness of these elements increases when they are nano sized. Nanotechnology is one of the major control methods used against pathogens due to its significant properties (16). It is considered an environmentally friendly approach that is safe and highly effective. Many studies have confirmed the role of nano-size elements in inhibiting the growth of pathogens and reducing disease severity in plants. Therefore, this study aimed to determine the cause of Dracaena plant wilt and control it using ordinator and nano-form nutrients.

## Materials and Methods

Fungal isolation and identification: samples were collected from Dracaena plant roots showing symptoms of vascular wilt from several nurseries in Baghdad. They were placed in polyethylene bags and brought to the laboratory for pathogen isolation. The samples were washed with water, and the pathogen was isolated from the roots of

infected plants by cutting root pieces of about 0.5 cm in length. These pieces were surface sterilized using a 1% sodium hypochlorite solution for two minutes and then rinsed thrice with sterile distilled water. The pieces were dried using sterile blotting paper and transferred to plastic dishes containing a potato dextrose agar (PDA) medium, which had been prepared and sterilized using an autoclave at 121 °C and 1.5 kg/cm<sup>2</sup> pressure for 15 min. Streptomycin sulfate was added at a rate of 50 mg/L as an antibiotic. Three plant pieces were placed per dish, with three replicates for each sample. The dishes were incubated at 25 ± 2 °C for three days. The isolated fungi were purified using the single spore method and identified morphologically based on the taxonomic keys of Leslie and Summerell (19).

**Pathogenicity Testing of Fungal Isolates:** A 2% agar water medium was prepared and sterilized in an autoclave at 121 °C and 1.5 kg/cm<sup>2</sup> pressure. The medium was poured into 9 cm Petri dishes. After solidification, a 0.5 cm disc was taken from the edge of a 5-day-old fungal colony of each isolate and inoculated onto the center of the dish. The plates were incubated at 25±2 °C for 3 days. Sterilized radish seeds were then planted 1 cm away from the edge of the fungal colony. The seeds were surface sterilized using a 1% sodium hypochlorite solution (commercial product) for 3 min, then thoroughly rinsed with sterile distilled water and dried on blotting paper. Fifteen seeds were planted per dish, and the experiment was conducted with three replicates for each isolate. Three control plates were also included, where only the seeds were planted without fungal inoculation. The dishes were incubated at 25±2 °C for 7 days. Disease severity was calculated according to (29), and the germination percentage was based on the following formula:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Number of planted seeds}} \times 100$$

**Testing the Pathogenicity of the Most Pathogenic Isolate in the Laboratory:** The pathogenicity of the fungal isolates was tested on *Dracaena* leaves in the laboratory. Healthy leaves were surface-sterilized using 1% sodium hypochlorite (containing free chlorine) for 2 minutes, then rinsed with sterile distilled water and left to dry. The leaves were cut into 8-cm-long pieces and placed in Petri dishes containing three sterilized and moistened filter papers at the base of the dish. A 0.5 cm disc was taken from the edge of a 5-day-old fungal colony and placed in the center of the leaf piece. The leaves were incubated at 25±2 °C for 10 days, with the filter papers moistened as needed. The experiment was conducted with four replicates, and the average area colonized by the fungus on the leaf was measured.

**Molecular Identification of the Pathogenic Isolate:** The DNA of the pathogenic fungal isolate was extracted using a specialized commercial kit from Bioneer, following the company's recommendations. After confirming the genetic material's purity, the pathogen's DNA was amplified using polymerase chain reaction (PCR) technology. This was done based on the ITS region using general primers (ITS1, ITS4). The PCR amplification results were sent to Macrogen, a South Korean company, for nucleotide sequence identification. The obtained sequences were compared using the BLAST program on the NCBI website (<http://www.ncbi.nlm.nih.gov>), and were deposited in GenBank.

Evaluating the Inhibitory Potential of Nanoparticles and Ordinator Oxides Against the Most Pathogenic Fungal Isolate: the effectiveness of ordinator and nano-sized copper oxide and iron oxide particles on the growth of the fungal isolate was tested under laboratory conditions using the poisoned food technique. The copper and iron oxides were prepared at 1%, 2%, and 3% concentrations in the sterilized potato dextrose agar (PDA) medium. The medium was sterilized using an autoclave, and streptomycin sulfate was added at 50 mg/L. The medium was poured into 9cm Petri dishes before solidifying and a 5-mm disc from the edge of a 5-day-old fungal colony was placed at the center of each dish. Three replicates, and a control (without additives), were used for each treatment. The inoculated dishes were incubated at  $25 \pm 2$  °C until the fungal colony in the control treatment fully covered the dish. The diameters of the fungal colonies were measured, and the inhibition percentage calculated according to (17).

The effectiveness of nanoparticles and ordinator oxides on fungal growth on *Dracaena* leaves, ordinator and nano-sized copper oxide, and iron oxide at 3% concentration in inhibiting the growth of the pathogenic fungus was tested. Healthy *Dracaena* leaves were surface sterilized using 1% sodium hypochlorite (free chlorine) rinsed with sterile distilled water, and left to dry. Leaf pieces of 8-cm length, were placed in the Petri dishes containing three sterilized and moistened filter papers at the base of each dish. A 0.5-cm disc from the edge of a 5-day-old fungal colony grown on PDA medium was placed in the center of each dish of the *Dracaena* leaf.

The dishes were incubated at  $25 \pm 2$  °C for 24 hours, after which the inoculated *Dracaena* leaves were sprayed until wet with ordinator copper oxide, nano copper oxide, ordinator iron oxide, or nano iron oxide, each in separate treatments. Each treatment was replicated three times. A control treatment was included, where leaves were inoculated with the fungus and sprayed with sterilized water instead of the test substances. The area colonized by the fungus and the percentage of the damaged area of the leaf were calculated 10 days after incubation. The formula used to calculate the percentage of the damaged leaf area was based on (7).

$$\text{Percentage of damaged leaf area} = \frac{\text{Area occupied by the fungus}}{\text{Total leaf area}} \times 100$$

Control efficiency was calculated using:

$$\text{Control efficiency} = \frac{\text{Percentage of damaged leaf area in the treatment}}{\text{Percentage of damaged leaf area in the control}} \times 100$$

### Results and Discussion

**Fungus Isolation and Identification:** The 11 fungal isolates from the *Dracaena* plants showing symptoms of vascular wilt were characterized by white colonies with a pinkish hue. They produced microconidia, macroconidia, and chlamydospores on the PDA medium.

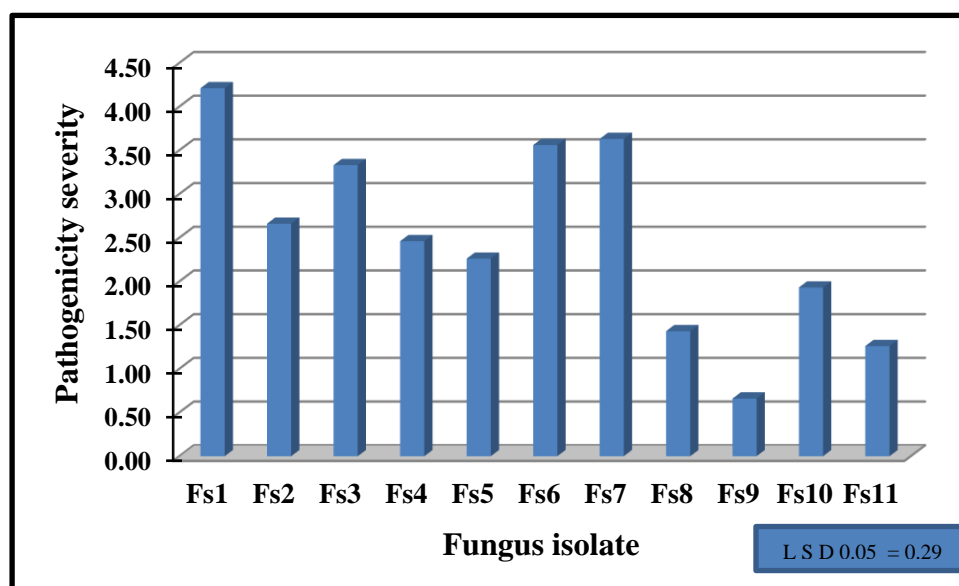
These morphological and microscopic characteristics matched those of fungal isolates belonging to *Fusarium oxysporum*.



**Figure 1: (a) Fungal growth on PDA medium; (b) microconidia and macroconidia (Scale bar: 20  $\mu$ m).**

**Pathogenicity Testing of Fungal Isolates:** Figure 2 shows that the tested fungal isolates exhibited varying degrees of pathogenicity, ranging from highly to weakly virulent, according to (29), on radish seedlings. The results indicated that the isolate Fs1 had the highest pathogenicity, with a severity score of 4.21, classifying it as highly virulent. In contrast, isolates Fs3, Fs6, and Fs7 were pathogenic, with severity scores of 3.33, 3.56, and 3.63, respectively, while the remaining isolates exhibited moderate to weak pathogenicity.

The differences in isolates pathogenicity may be due to their varying ability to rapidly produce enzymes that inhibit seed germination or cause seed decay. The quantity of enzymes secreted by the isolates also influences seed germination and decay. According to (14), fungal isolates can produce enzymes such as cellulases, cutinase, pectinase, and proteases, in addition to secreting toxins like fusaric acid, fumonisins, lycomarasmin, and dehydrofusaric acid, which affect cell viability and enhance the fungus's virulence (31).

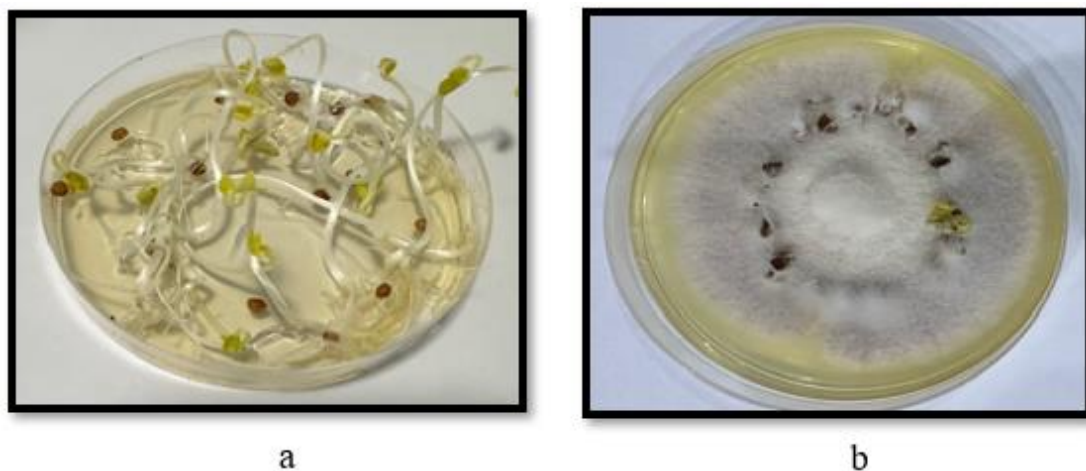


**Figure 2: Pathogenicity of *F. oxysporum* isolates according to the Sneh scale.**

Table 1 shows that the *Fusarium oxysporum* isolates exhibited varying pathogenic abilities regarding seed germination, ranging from 55.53% to 97.76%, compared to the



control's 100%. The Fs1 isolate had the highest ability to reduce seed germination, at a rate of 55.53%, while isolate Fs10 recorded 97.76%, not significantly different from Fs11, Fs8, and the control treatment, which achieved 100% germination (Figure 3).



**Figure 3: Pathogenicity test on radish seeds. (a) Control treatment, (b) Fungal treatment.**

**Table 1: Pathogenicity test for *Fusarium* spp isolates.**

Isolate number	Isolate symbol	Seed germination after 7 days (%)
1	Fs1	55.53
2	Fs2	86.63
3	Fs3	77.76
4	Fs4	86.63
5	Fs5	82.2
6	Fs6	62.16
7	Fs7	62.2
8	Fs8	88.86
9	Fs9	86.63
10	Fs10	97.76
11	Fs11	91.1
12	Control	100
LSD at 0.05		15.772

Each value represents the meaning of three replicates.

Pathogenicity of the Isolates in the Laboratory: The test (Figure 4) showed significant isolate pathogenicity on the *Dracaena* leaves in Petri dishes, with significant differences over the control treatment. The average affected area for the most virulent isolate, Fs1, reached 1.24 cm<sup>2</sup>, while that for the control treatment was 0 cm<sup>2</sup> (Figure 5). This could be attributed to the fungi's ability to produce various enzymes and possibly toxins that break down plant cell walls and infect them.

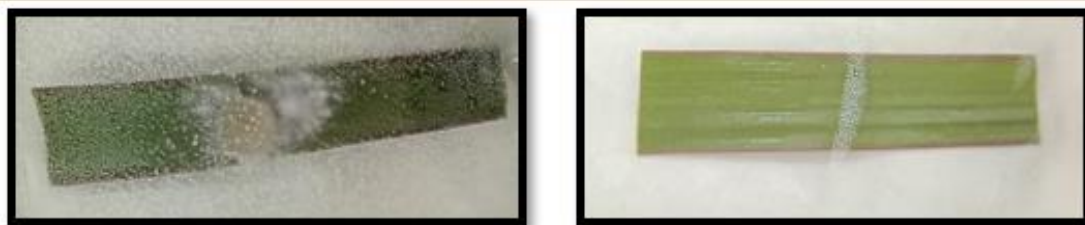


Figure 4: Spread of infection in the affected area.

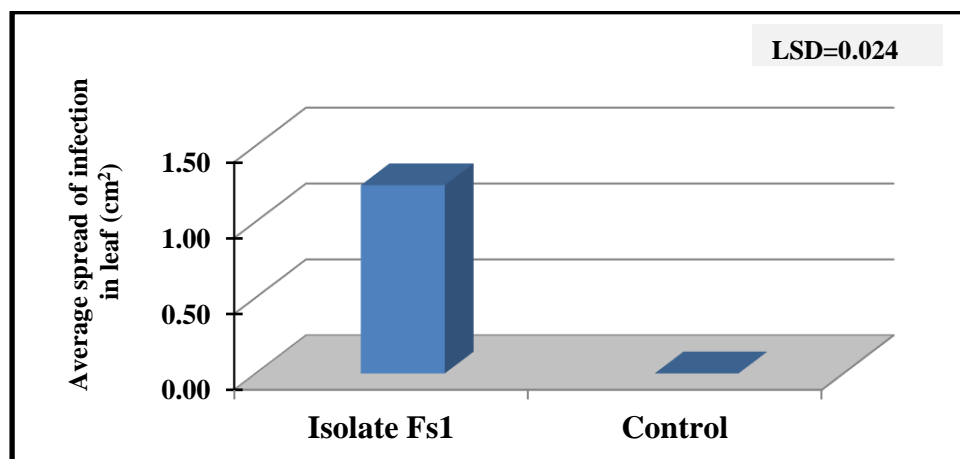


Figure 5: Average spread of infection in the affected leaf area.

**Molecular Identification of the Pathogenic Isolate:** Molecular analysis, based on a pair of general primers, ITS1/ITS4, for identifying the pathogenic fungal isolate showed the presence of a band on an agarose gel with an estimated molecular weight of 500 bp. The nucleotide sequence analysis indicated a 100% match with global isolates from the NCBI GenBank belonging to *Fusarium oxysporum*. The nucleotide sequences of the fungal isolate responsible for Dracaena wilt disease were deposited in the GenBank under the accession number PP273503.

**Inhibitory Potential of Nanoparticles and Ordinator Oxides Against the Most Pathogenic Fungal Isolate.** The antagonistic potential test for ordinator and nano iron oxide, as well as ordinator and nano copper oxide, at concentrations of 1%, 2%, and 3%, using the poisoned food technique, showed apparent effectiveness in inhibiting the growth of the pathogenic fungus (Table 2). The lowest fungal growth rate (0 cm) was in the nano iron oxide treatment at the 3% concentration, with a 100% inhibition rate. At 2% and 1% concentrations, the rates were 0.3 cm and 0.36 cm, respectively, with 96.6% and 95.8% inhibition rates. In contrast, the nano copper oxide treatment at concentrations of 3%, 2%, and 1% recorded fungal growth rates of 1.03 cm, 1.43 cm, and 1.56 cm, respectively, with inhibition rates of 88.4%, 84.0%, and 82.5%.

The growth rates for the ordinator iron oxide and ordinator copper oxide treatments were 0.56 cm and 2.06 cm, respectively, at a 3% concentration, with inhibition rates of 93.6% and 77.0%. In contrast, the control treatment (fungus alone) recorded a growth rate of 9 cm, with a 0% inhibition rate. These results are consistent with (4), who confirmed the role of nano iron particles in inhibiting the growth of *Alternaria mali*, *Diplodia seriata*, and *Botryosphaeria dothidea*, with an inhibition rate of 84%. (13) reported the effectiveness of different concentrations of nano copper in inhibiting the growth of *Fusarium kuroshium*, achieving an inhibition rate of up to 80%. (15) showed

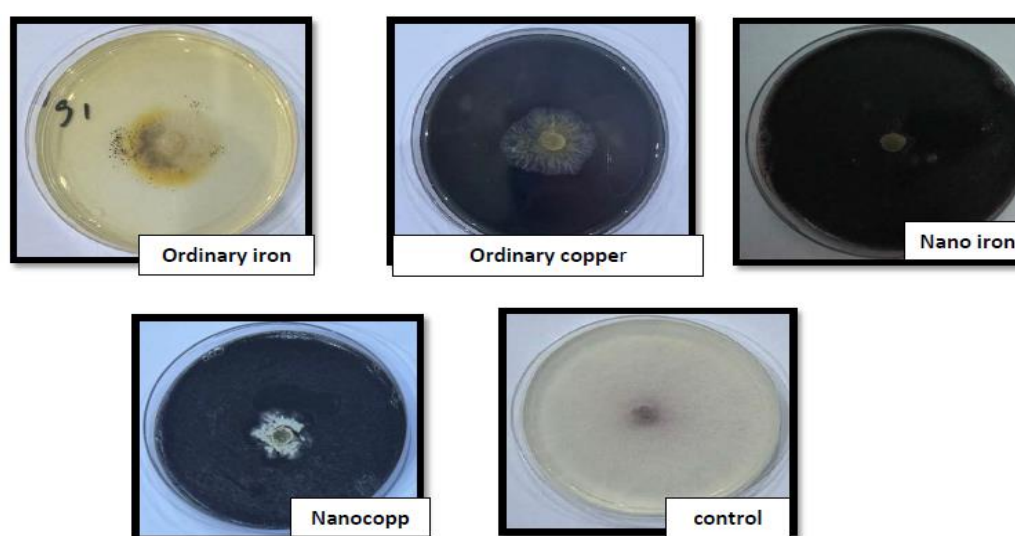


the effectiveness of nano copper oxide ( $\text{Cu}_2\text{ONPs}$ ) at a concentration of  $100 \text{ mg L}^{-1}$  in inhibiting *Fusarium solani*, with an inhibition rate of 74.44%. (33) reported that nano copper particles inhibited the growth of *F. oxysporum* by 100% at a concentration of 80 ppm. (28) highlighted the role of both the ordinator and nano copper particles in inhibiting the growth of the brown rot pathogen on potatoes (*Ralstonia solanacearum*), with growth measured at 3.6 colony-forming units (cfu)  $\text{mL}^{-1}$  compared to the control's 9.8 cfu  $\text{mL}^{-1}$  at  $1 \text{ mg mL}^{-1}$  concentration. The inhibition zone was 19.3 mm when using nano copper particles. (11) noted that nano copper oxide inhibited the growth of *Fusarium solani* in Petri dishes at 50, 100, and 250 mg/L concentrations and inhibition rates of 46.30%, 71.36%, and 91.17%, respectively, compared to the control's 0%.

**Table 2: Effectiveness of nano iron oxide, nano copper oxide, iron oxide, and copper oxide in inhibiting the growth of pathogenic fungus using the poisoned food technique.**

Treatment	Concentration (g/100ml)	Average growth of two perpendicular diameters (cm)	Inhibition rate (%)
Ordinary copper oxide ( $\text{Cu}_2\text{O}$ )	1	2.9	67.7
	2	2.46	72.5
	3	2.06	77.0
Nano copper oxide	1	1.56	82.5
	2	1.43	84.0
	3	1.03	88.4
Ordinary iron oxide ( $\text{FeO}$ )	1	3.66	59.2
	2	3.1	65.5
	3	0.56	93.6
Nano iron oxide	1	0.36	95.8
	2	0.3	96.6
	3	0	100
Control		9	0
LSD at 0.05		0.172	1.9101

Each value represents the meaning of three replicates.



**Figure 6: Comparative antifungal efficacy of nano and ordinary oxides in inhibiting the *Fusarium oxysporum* fungus.**

Effectiveness of Nano and Ordinator Oxides on Fungal Growth on Dracaena Leaves: The tests for the effectiveness of nano and ordinator oxides in inhibiting fungus on Dracaena leaves (Table 3) showed the nano iron oxide and nano copper oxide treatments completely preventing fungal growth. Damaged leaf area and the percentage of the affected area were 0% at the 3% concentration of the materials applied. In contrast, the fungal-affected areas for the ordinator iron oxide and copper oxide treatments were 70.91 mm<sup>2</sup> and 75.88 mm<sup>2</sup>, respectively, with damaged leaf areas forming 8.864% and 9.485%. The control treatment showed fungal-affected and damaged-leaf areas at 235.5 mm<sup>2</sup> and 29.43%, respectively.

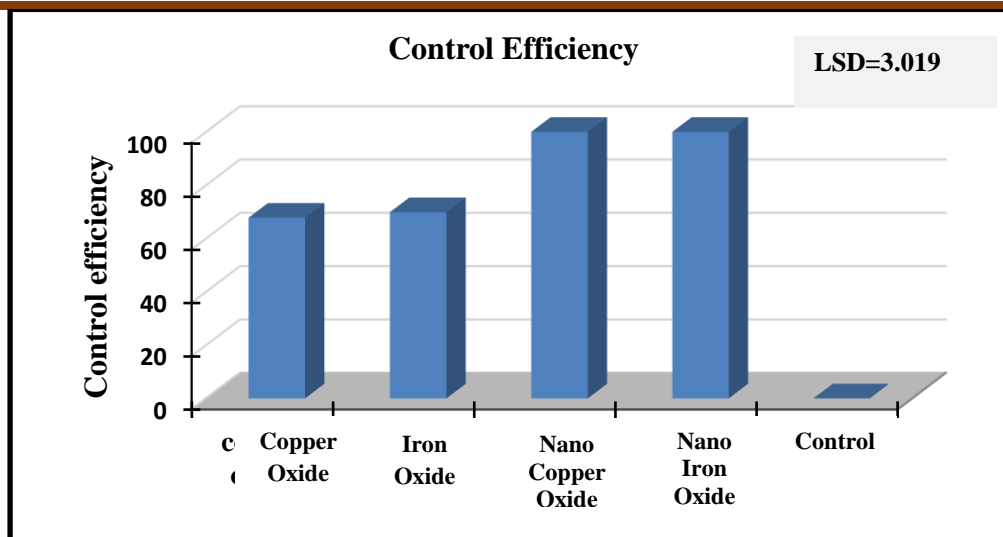
**Table 3. Effectiveness of nano iron oxide, nano copper oxide, iron oxide, and copper oxide on the growth of the *Fusarium oxysporum* fungus**

Treatment	Fungal-affected area (mm <sup>2</sup> )	Damaged leaf area (%)
Ordinator copper oxide	75.883	9.485
Nano copper oxide	0	0
Ordinator iron oxide	70.91	8.864
Nano iron oxide	0	0
Control	235.5	29.438
LSD at 0.05	7.10	0.88

Each value represents the meaning of three replicates

Figure 7 shows that control efficiency reached 67.77% and 69.88% in the treatments with copper oxide and iron oxide, respectively, while 100% efficiency was recorded for the nano copper oxide and nano iron oxide treatments compared to the control treatment's 0%. These results align with nanoparticles exhibiting antimicrobial properties, enabling them to control diseases (25). (9) demonstrated a reduction in disease severity and enhanced plant protection by 82.15% when using nano iron oxide at 20 µg mL<sup>-1</sup> concentration against *F. oxysporum* in tomatoes, along with increased photosynthetic pigments and antioxidant enzymes. (28) found that nano copper particles reduced brown rot disease in potatoes by 71.2%, compared to 43.0% for ordinator copper particles. (2) reported the effectiveness of nano iron particles in reducing the severity of Fusarium wilt and increasing plant height, dry and fresh weights, and the number of pods in broad beans. (23) noted the effectiveness of nano iron oxide particles at a concentration of 20 µg mL<sup>-1</sup> in reducing the infection rate of *Fusarium oxysporum* in eggplant from 82.5% to 22.5%.

The role of nanoparticles in inhibiting fungal growth is attributed to their small size, which causes cell damage by interfering with the absorption of Ca<sup>2+</sup> ions within the cells, increasing internal cellular disruption, penetration, and cell damage (20). Metallic ions enter the microbial cell membrane, affecting electrostatic interactions, which leads to damage to the cell membrane and internal organelles (30).



**Figure 7: Control efficiency on Dracaena leaves using nano and ordinator oxides on the growth of the Fusarium oxysporum fungus.**

### Conclusions

This study confirms *Fusarium oxysporum* as the causal agent through robust morphological and molecular characterization. Both conventional and nanoparticle forms of iron oxide (FeO) and copper oxide (Cu<sub>2</sub>O) exhibited significant antifungal activity, with nano FeO achieving 100% inhibition at 3% concentration, outperforming nano Cu<sub>2</sub>O. In planta leaves trials demonstrated complete suppression of fungal lesions under nano treatments, in contrast to conventional oxides. Dracaena leaves applicability was further evidenced by disease control, underscoring the potential of nano oxides as sustainable alternatives to chemical fungicides. These findings advocate for scaled validation of nanoparticle-based formulations in integrated pest management.

### Supplementary Materials:

No Supplementary Materials.

### Author Contributions:

Eman K. Abdul-Karim: methodology, writing—original draft preparation; Tariq. A. Kareem and Halima Z. Hussein: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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### Institutional Review Board Statement:

The authors confirm that no ethical approval was necessary for this work, as it complied with institutional and national guidelines for plant-based studies exempt from IRB oversight.

### Informed Consent Statement:

Not applicable.

### Data Availability Statement:

Data available upon request.

### Conflicts of Interest:

The authors declare no conflict of interest.

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