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Evaluation of chromium nanoparticles against *Fusarium oxysporum* the causal pathogen of strawberry wilt disease

Hussein M. A. M.[a*], Hassan S. H.[b,c]

[a]*Department of Plant Pathology, Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt*
[b]*Biology Department, College of Science, Sultan Qaboos University, 113 Muscat, Oman*
[c]*Botany and Microbiology Department, Faculty of Science, New Valley University, 72511 New Valley, Egypt*

Abstract

Strawberry became one of the most important fruit crops in last Decade. Strawberry infected with many pathogens and one of the most important pathogens in Egyptian fields is wilt disease. In this study there are twenty isolates of wilted strawberry. The causal pathogen isolates identified as *Fusarium oxysporum.* Pathogenicity tests carried out with the isolated strains of *F. oxysporum* shown wilt symptoms. Strawberry used for symptoms incidence *F. oxysporum and* revealed wilt disease; chromium nanoparticle was evaluated under greenhouse conditions, Uniform fungicide used as standard control. Chromium nanoparticle shown success results in controlling the wilt causal pathogen *Fusarium oxysporum*.

Keywords: strawberry, *Fusarium oxysporum*, nanoparticles, wilt.

*Corresponding author: Hussein M. A. M.,
E-mail address: mmon3m@gmail.com

1. Introduction

Fusarium oxysporum fragariae is the causal pathogen of strawberry wilt rise losses in commercial strawberry production to noticeable levels, the pathogen which caused strawberry wilt to increase the rate of losses in commercial strawberry production to a point where they are significant (Juber *et al*., 2014). *F. oxysporum* is the causal pathogen for wilt disease occurred in strawberry (Gueevara *et al*., 2020). *F. oxysporum* is the cause of wilting signs in strawberry fields, and it can be used to diagnose Fusarium wilt, a disease that is spreading in many countries (Fang *et al*., 2011; Koike *et al*., 2009). *Fusarium oxysporum* f.sp. *fragariae* the causal pathogen of wilt disease in strawberry is a key loss of strawberry production (Henry *et al*., 2017). The occurrence of *F. oxysporum* f.sp. *fragariae* in strawberry in Turkey is the principle causal pathogen of wilt (Benlioglu *et al*., 2014). Michielse *et al*. (2009) mentioned that *Fusarium oxysporum* f.sp. *fragariae* cause death of strawberry because it speeds up the wilting in strawberry (Fang *et al*., 2012). Cultivars Festival, Florida and Sweet Charlie of strawberry were varied in their resistance and susceptibility under field and green house conditions when it infected with the fungal causal pathogen *F. oxysporum* (Essa, 2015). The antibacterial activity of NPs is due to their interaction with functional groups on the surface of microorganism cells, which causes the microorganism to be eliminated (Choi, *et al*., 2008). Lipșa *et al*. (2020) Evaluated the antifungal activity nanoparticles on *Fusarium oxysporum* and their fungicidal activity. Different methods used to control Fusarium wilt in strawberry such as in soil biocides application on varied cultivars before infection (Fravel *et al*., 2003). This study aimed to survey on strawberry against fungal pathogen *Fusarium oxysporum* f.sp. *fragariae* the causal of wilt disease under greenhouse conditions. Evaluate the ability of nanoparticles compounds to control the strawberry wilt disease.

2. Materials and methods

2.1 Isolation and identification

Collected samples of wilted strawberry from El-Khatatba territory, Giza, Egypt infected plants were rinsed under running tap water before being dried, dipped in 1% sodium hypochlorite solution for 3 minutes, then being washed three times with sterile distilled water and dried on sterile filter papers. The samples were chopped for small pieces then aseptically placed onto medium Potato dextrose agar PDA in Petri dishes. Petri dishes are incubated at 27±2 ºC and every day checked. Fungal colonies hyphal tip technique used (Hawker, 1956) then purified on new PDA medium Petri dishes. Identification of the isolated fungi carried out according to their morphological characteristics (Booth, 1977; Leslie and Summerell, 2008; Nirenberg and O'Donnell, 1998). Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt, verified the identification.

2.2 Pathogenicity tests

The pathogenicity of *Fusarium oxysporum* was assessed as described by Ahmed (2005). Strawberry transplants were evaluated 15 days after inoculation. Each treatment included three replicates, with three pots per replicate. Disease severity was calculated using the following equation:

$$DS\% = \Sigma d \,/\, (d\_max \times n) \times 100$$

Where: d = Disease rating of each plant. d_max = Maximum possible rating. n = Total number of plants per replicate.

Wilt disease severity was recorded 30 days after transplanting, following the method described by Fang et al. (2012b). *F. oxysporum* was cultured under greenhouse conditions in pots with a 30 cm diameter, each filled with 7 kg of sterile sandy/clay soil mixed at a 1:1 ratio. Three strawberry plants were transplanted into each pot, with each pot representing one replicate. Three replicates were used for each treatment. The severity of wilt disease was assessed 30 days post-transplanting.

2.3 Nanoparticles synthesis

2.3.1 Preparation of fungal filtrate

The fungal strain *Fusarium oxysporum* was cultivated aerobically in 500 mL Erlenmeyer flasks containing 200 mL of Capek-Dox liquid medium, following the method described by Thom and Church (1926). The mixture was incubated for 96 hours at 32°C and then filtered under vacuum using Whatman No. 2 filter paper.

2.3.2 Zinc oxide nanoparticles biosynthesis

The fungal filtrate was treated with an equal volume of zinc sulfate heptahydrate solution (1 mM) and incubated for 48 hours at 32°C. White precipitates, indicating the biosynthesis of zinc oxide nanoparticles (ZnO NPs), were observed at the bottom of the flask. These precipitates were separated by centrifugation at 10,000 rpm for 10 minutes and stored for further analysis.

2.3.3 Chromium nanoparticles synthesis

Chromium nanoparticles (Cr NPs) were synthesized using chromium oxide supplied by Alpha Chemika, India, and *Rosmarinus officinalis* (rosemary) leaf extract, based on the biosynthesis methods described by Baskar *et al*. (2013) and Farghaly and Nafady (2015).

2.3.4 Rosmarinus officinalis leaf extract Preparation

Fresh rosemary leaves (*Rosmarinus officinalis* L.) were washed thoroughly with sterilized distilled water. Five grams of leaves were boiled in 100 mL of distilled water for 15 minutes in a sterilized conical flask. The solution was cooled and filtered using Whatman filter paper. The filtrate was centrifuged at 4,000 rpm for 5 minutes, and the

supernatant was used for Cr NP biosynthesis.

2.3.5 Nanoparticles characterization

The nanoparticles were characterized using UV-Visible spectrophotometry (JENWAY 7315), scanning electron microscopy (SEM, JSM5400LV), and transmission electron microscopy (TEM, JEM100CXII) to determine their morphology and particle size.

2.4 In vitro assessment of antifungal activity of nanoparticles

The antifungal activity of nanoparticles was evaluated against *Fusarium oxysporum*, the causal agent of strawberry wilt, using the well-diffusion method described by Shameli *et al*. (2012) and Khalil (2013). The fungal inoculum was prepared from an isolate with the highest disease severity index, adjusted to 1×10^6 cfu/mL according to McGuire and Kelman (1984). Sterilized Petri dishes were filled with nutrient sucrose agar, allowed to solidify, and streaked with fungal suspension. Sterilized discs immersed in nanoparticle solutions of known concentrations were placed on the plates, which were incubated at 28±2°C for 7 days. The inhibition zone diameters were measured in millimeters. Each treatment included four replicates, and the experiment was repeated twice.

2.5 Control of strawberry wilt using nanoparticles under greenhouse conditions *in vivo*

This experiment evaluated the *in vivo* efficacy of nanoparticles in controlling strawberry wilt. Treated plants were immersed in 2000 µg/mL of chromium nanoparticle solution for 30 minutes, air-dried, and transplanted. Control plants were treated with sterilized distilled water. Observations were made over 30 days, following the method of De Boer and Kelman (1978).

2.6 Efficacy of nanoparticles against strawberry wilt disease under greenhouse conditions

The efficacy of nanoparticles in controlling *Fusarium oxysporum* was assessed under greenhouse conditions. Treatments included positive controls (infected plants) and negative controls (healthy plants). Replicates were prepared as described earlier. The experiments were conducted at the Plant Pathology Research Farm and Greenhouse, Assiut University, Egypt.

2.7 Biochemical assessment of peroxidase activity

Peroxidase activity was measured using the method described by Jockusch (1966). The assay mixture included sodium phosphate buffer, and control tubes were prepared by replacing the sample extract with buffer. Absorbance was recorded at 546 nm, and peroxidase activity was expressed in U/mL·min after 3 minutes.

2.8 Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), and means were compared using the least significant difference (LSD) test at a significance level of $P \leq 0.05$, as described by Gomez and Gomez (1984).

3. Results

3.1 Isolation and identification

Seven fungal isolates were obtained from wilted roots of strawberry plants collected from two locations within the El Khatatba region of Giza Governorate, Egypt. These isolates were morphologically and microscopically identified as *Fusarium oxysporum* Schlecht.

3.2 Pathogenicity tests

Data in Table (1) indicate that *Fusarium oxysporum* exhibited the ability to induce wilt disease symptoms in strawberry plants. *Fusarium oxysporum* isolate 1 caused a disease severity of 55.3%, while *Fusarium oxysporum* isolate 2 resulted in a disease severity of 45.4%.

Table (1): Disease severity of *Fusarium oxysporum* on strawberry.

Isolates	Disease severity (%)
Fusarium oxysporum 1	55.3
Fusarium oxysporum 2	45.4
Fusarium oxysporum 3	41.2
Fusarium oxysporum 4	12.8
Fusarium oxysporum 5	16.2
Fusarium oxysporum 6	18.4
Fusarium oxysporum 7	18.4
L.S.D. ≥ 0.05	3.22

3.3 Control of strawberry wilt disease with Fusarium oxysporum using nanoparticles

3.3.1 In vitro assessment

Data in Table (2) revealed that all nanoparticles exhibited varying levels of inhibitory activity against *Fusarium oxysporum*, the causal agent of strawberry wilt disease, using the fungicide Uniform as the standard control. The assessment was based on the estimation of growth diameter, which served as an indicator of fungal growth reduction. Among the nanoparticles tested, chromium nanoparticles showed the most significant inhibitory effect at a concentration of 2000 ppm, with a fungal growth diameter of 7.0 mm. In comparison, Uniform demonstrated the highest antifungal activity against the pathogen, achieving optimal effectiveness at a concentration of 2500 ppm.

Table (2): Effects of chromium NPs on *Fusarium oxysporum* radial growth *in vitro*.

Concentrations (µg/ml)	*F. oxysporum* growth diameter (mm)
Cr NPs 2000	7.0
Cr NPs 1000	12.0
Cr NPs 500	68.0
Uniform 2500 ppm (Positive control)	2.0
Uninfected control	100.0
L.S.D. ≥ 0.05	2.9

3.3.2 In vivo assessment under greenhouse conditions

The nanoparticles significantly suppressed strawberry wilt disease caused by *Fusarium oxysporum*, as detailed in Table (3). Chromium nanoparticles (Cr NPs) at the highest concentration of 2000 ppm achieved a disease suppression rate of 58.9%, whereas the lowest reduction of 31.1% was observed at a concentration of 500 ppm. In comparison, the standard fungicide Uniform demonstrated complete efficacy, achieving 100% disease reduction.

Table (3): Effects of chromium NPs different concentrations on wilt disease under greenhouse conditions.

Concentrations (µg/ml)	DS (%)	D.R. (%)
Cr NPs 2000	41.1	58.9
Cr NPs 1000	56.4	43.6
Cr NPs 500	68.9	31.1
Uniform 2500 ppm (Positive control)	100	0.0
Uninfected control	0.0	100
L.S.D. ≥ 0.05	0.0	100

3.4 Biochemical analysis of different nanoparticles concentrations on peroxidase activity in vivo

3.4.1 Peroxidase enzyme estimation of strawberry plants

Data in Table (4) indicate that peroxidase activity, measured in U/ml.min after 3 minutes for each treatment, varied significantly between control and treated samples. The observed values were 0.314, 0.578, and 0.467, respectively, with the lowest activity recorded in healthy plants at 0.102 U/ml.min. These results highlight a notable difference in peroxidase enzyme levels among the treatments and control.

Table (4): Biochemical analysis of different nanoparticles concentrations on peroxidase activity *in vivo*.

Concentrations (µg/ml)	Peroxidase (U/ml.min)
Cr NPs 2000	0.314
Cr NPs 1000	0.246
Cr NPs 500	0.467
Uniform 2500 ppm (Positive control)	0.578
Uninfected control	0.198
L.S.D. ≥ 0.05	0.102

4. Discussion

Fusarium oxysporum Schlecht, were isolated from the roots of diseased strawberry plants collected from some Farms in Khatatba territory Giza Governorates. These results of the pathogenicity tests are confirmed by the findings by Zhao *et al*. (2009) and Suga *et al*. (2013), that *F. oxysporum* f.sp. *fragariae* was identified as the predominant pathogen in many countries in the world. Also, Williamson *et al.* (2012) reported that the main causal agent of strawberry wilt was *F. oxysporum* that isolated from roots in South Carolina. Several other studies indicated that *F. oxysporum* was among the major causal agents of strawberry wilting (Ebihara and Uematsu, 2014; Nagarajan *et al*., 2006). The presented study showed that strawberry infected with to Fusarium wilt disease this results in agreement with those obtained by (Essa, 2015; Fang, *et al*., 2012). The study was carried out to investigate the *in vitro* and *in vivo* antifungal activities of two tested NPs in management with strawberry wilt disease caused by *F. oxysporum*, data illustrated that Cr NPs synthesis and characterization are in match with Farghaly and Nafady (2015). Antifungal activity *in vitro* of the nanoparticles of Cr NPs caused marked suppression against strawberry wilt disease caused by *F. oxysporum*. according to the findings. According to Kim *et al*. (2012), antioxidant system of strawberry plants revealed and detected peroxidase by Cr NPs and treatments. The nanoparticles gave activity of peroxidase but less than what revealed with the infected control treatment with *F. oxysporum* that induced the peroxidase enzyme activity. This rise in PPO activity can be proposed that Cr NPs activated the move in plant metabolism towards the augmented synthesis of phenolics hence giving more antioxidant and defense against Cr NPs within high PPO activity (Bayat *et al*., 2019; Lipșa *et al*., 2020; Xie, 2016).

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