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Antifungal activity of fabricated mesoporous silica nanoparticles against early blight of tomato

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ABSTRACT

There is a growing interest in the development of alternative strategies in plant disease management to reduce dependency on synthetic chemicals. In this study, we described synthesis and evaluation of the direct antifungal activity of mesoporous silica nanoparticles (MSN) compared to metalaxyl (recommended fungicide) against *A. solani* under laboratory and greenhouse conditions. The structural features of MSN such as high porosity, small particle size and suitable shape contributed to its high antifungal efficacy against *Alternaria solani*. Laboratory synthesized MSN showed marked increase in tomato growth parameters compared to untreated control. Our study presents promising results of the use of MSN as an effective and safe alternative of fungicides for managing tomato early blight.

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Introduction

Tomato (*Lycopersicon esculentum* L. H. Karst.) is an important vegetable crop worldwide. Tomato early blight caused by *Alternaria solani* is one of the most destructive diseases worldwide; yield losses of up to 80% have been attributed to this disease [1–3].

The control of tomato early blight mainly relies on the frequent use of synthetic fungicides. Numerous fungicides are potential compounds against this pathogen but these chemicals are not ideal long-term solutions because of the high cost, residues, and the impacts on the environment and human health [4–7]. Moreover, the evolution of resistance of plant pathogens such as *A. solani* against fungicides is a problem of major concern [8,9]. Therefore, safe, effective, and eco-friendly control agents are in demand [10]. Recently, the search for new control agents in pest management has become an urgent task. Nanotechnology can play significant role in this regard. The development of novel agents for detection and control of plant diseases are examples of the major contributions of nanotechnology to agriculture and food systems [11]. Nanotechnology can play several roles in the progress of available plant protection tools [12] and may be used in the control of plant pathogens in terms of control agents delivery or disease detection.

Mesoporous materials such as silica have widespread applications, i.e., in disease diagnosis and therapy [13–17]. Recently, the attention given to mesoporous silica is attributed to their unique characteristics, such as uniformed mesoporous tunnels, narrow pore size distribution, good biocompatibility, low toxicity, and chemical stability. Much effort has been devoted toward the improvement and manipulation of this material for various applications. In addition, the design of mesoporous and nanomaterials with engineered features, including geometrical shapes, framework matrices, compositions, and active-site functions, have important advantages in applications for medical and agricultural purposes.

In this study we described the synthesis and evaluation of mesoporous silica nanoparticles (MSN) with large, tunable, and open cylindrical pores as potential antifungal agent against *A. solani* under laboratory and greenhouse conditions. The efficacy of MSN was evaluated against tomato early blight as compared with the recommended fungicide, metalaxyl.

Materials and methods

Source of chemicals

Tetramethylorthosilicate (TMOS), dodecane (C12H26), and the triblock copolymers of poly(ethyleneoxide–b-propylene oxide–b-

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ethylene oxide) (Pluronic P123; EO20PO70EO20) were obtained from the Sigma-Aldrich Company, Ltd. (USA). These analytical-grade chemicals were used without further purification. Metalaxyl is the recommended fungicide for the control of tomato early blight pathogen; this chemical with a trade name of Metalaxyl 25% EC Nanjing Essence Fine-Chemical Co., Ltd. was obtained from the Agricultural Development Co., Ltd. (Cairo, Egypt).

Synthesis of MSN

The one-pot direct template approach was used to synthesize the MSN, as previously reported [18–20].

Characterization of MSN

A Belsorp Min-II analyzer was used to test the N2 adsorption–desorption isotherms at 77 K. Based on the Brunauer–Emmett–Teller (BET) theory, the specific surface area (*S*BET) was determined with multi-point adsorption data from the linear portion of the N2 adsorption isotherms. The cylindrical pore diameter was defined by Barrett–Joyner–Halenda (BJH) analyses. The small/wide angle powder X-ray diffraction (XRD) measurements of the fabricated material were conducted with a 18 kW diffractometer (Bruker D8 Advance) with monochromated Cu Kα irradiation. Transmission electron microscopy (TEM) micrographs were obtained with a 200 kV electron microscope (JEOL 2000 EX II). Field-emission scanning electron microscopy (FE-SEM) images were obtained with a Hitachi S-4300 microscope. Carbon tape was used as a substrate to fix the MSN powder on a SEM stage before insertion into the chamber. The 29Si MAS NMR spectra were obtained with a Bruker AMX-500 spectrometer.

Assessment of growth inhibition

The efficacy of MSN and metalaxyl were evaluated against *A. solani* under laboratory conditions. The efficacy was determined as the per cent of inhibition in the growth relative to the control treatment. Potato dextrose agar (PDA) medium was poured into Petri dishes with 15 ml per dish. One well was punched in the center of each plate after solidification. The plates were inoculated in the center with a disk (5 mm diameter) bearing the mycelium growth from the *A. solani* culture (5 days old culture). A 50 μl aliquot of MSN and metalaxyl, at concentrations of 100, 200, 300 and 400 mg/l, was added to the respective punched holes. A 50 μl aliquot of sterilized liquid medium was added into selected wells as the control. The plates were sealed with parafilm to reduce the evaporation of the tested materials. The incubation time for the plates at 28 °C was extended until the full growth of *A. solani* (mycelia reached the edge of the plate) in the untreated control. The formula by Vincent [21] was used to calculate the percentage of inhibition of *A. solani* as shown in Eq. (1). Each treatment was replicated three times and per replication five plates were maintained.

$$I\% = (A - B)/A \times 100 \quad (1)$$

where A is the fungal radial growth in the control, and B is the fungal radial growth in the treatment.

Preparation of spore suspension

Pathogenic *A. solani* isolated from infected tomato plant and identified in Plant Pathology Research Institute, Giza Egypt, was grown on potato dextrose agar for culturing. To enhance sporulation, cultures were exposed to fluorescent light (80 μmol/m^2/s) for 6 h daily prior to use. For each Petri dish, 10 ml of sterilized water was added and the conidia were collected using a sterilized brush. The spore suspension of the fungus was filtered through three layers of nylon mesh. The concentration of conidia was determined and adjusted to 10^6 conidia/mL with a hemocytometer.

Experimental design and treatments

The efficacy of MSN was studied in pots under greenhouse conditions at the Kafr El-Sheikh University Farm in Egypt for two growing seasons (2013/2014–2014/2015). Completely randomized design was used for this experiment with four replicates for accurate data. For each pot, 5 one-month-old tomato seedlings (GS13 variety) were transplanted (20 cm high; 25 cm diameter) filled with sterilized soil. After two weeks of transplanting, tomato seedlings (45 days old) were inoculated with *A. solani* as foliar spray with a spore suspension of 10^6 conidia/ml [22]. The inoculated plants were covered with plastic bags for 48 h to maintain the high relative humidity and support fungal infection [23]. After one week of incubation, the respective growing seedlings were sprayed with MSN and metalaxyl at concentration levels of 200 and 400 mg/l using hand atomizer. Tomato seedlings were sprayed twice with 10 days intervals. Control treatment was sprayed with water only. Disease severity was determined after 10 days of last spray. The scale by [24] was used to calculate disease severity. Plant height, fresh and dry weight were measured after 10 days of the last spray to evaluate the effect of applied treatments on tomato growth parameters.

Statistical analysis

Statistical analysis for the data was performed with JMP software version 8 using the Turkey Kramer HSD test for determining significant differences among treatment at P = 0.05 level.

Results

Characterization of the fabricated MSN antifungal agent

The SA-XRD pattern of MSN antifungal agent is shown in Fig. 1A. This pattern reflects the well-ordered structure of the fabricated MSN, with the well-resolved diffraction peaks and characteristic SA-XRD patterns of the Ia3d symmetry with a highly ordered mesostructure. The SA-XRD pattern showed the well-defined (2 1 1), (2 2 0), (4 0 0), and (3 3 2) diffraction planes that are features of highly ordered cubic Ia3d nanophase domains (Fig. 1A).

The pore size distribution of cubic Ia3d silica MSN was examined by N2 adsorption isotherms (Fig. 2). The isotherm exhibited typical type-IV sorption with the typical H1 hysteresis loop of characteristic cylindrical mesoporous materials [19,25]. The analysis of the adsorption isotherms with the BET method revealed that the *S*BET of silica was 489 m2/g, the *V*P was 0.69 cm^3/g, and the *D*P was 10.7 nm.

Key features of this material design include the high level of 3D arrangement, nano-sized particle morphology, and uniform mesoporous distribution of the target into the mesoporous surface architectures, as proven by analyzing the TEM and SEM micrographs, XRD patterns, and N2 isotherm profiles (Figs. 1–3). The TEM images of cubic *Ia3d* silica monoliths were recorded along the [3 1 1] direction (Fig. 2A) and showed the well-defined and regulated mesopore channels that were harmonized along all directional configurations. The insert in Fig. 2A is the corresponding ED pattern analysis, which reveals that the formation of ordered cubic Ia3d lattice symmetry of the silica monolith is congruous with the well-defined XRD patterns. FE-SEM micrographs of the silica monoliths demonstrated the stable morphologies of the

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