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.

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-
ethylen 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–Te ller (BET) theory, the specific surface area (SBET) 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 radiation. 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.

\[
\text{I%} = \frac{(A - B)}{A} \times 100
\]

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/m²/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 10⁶ 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 10⁶ 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 monolith 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 SBET of silica was 489 m²/g, the VP was 0.69 cm³/g, and the DP 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
molecules for all cases of calcined and crushed monoliths. In addition, large-sized particles with diameters of 20–150 μm were present (Fig. 2B–D). Remarkably, the monoliths in micrometer-sized particles were a result of the aggregation of large amounts of nanoparticles. The developed MSN featured such as 3D cubic Ia3d structures, with cylindrically-shaped and uniform pore sizes, as well as the conversion of monodispersed meso-/macroporosities into ultra- or micrometer-sized particles (≥150 μm). These features probably increased its efficiency as a potential antifungal agent against early blight disease of tomato. Fig. 3 showed the three resolved signals of the monolithic cubic Ia3d silica sample at the chemical shift (δ) of −85, −98, and −106 ppm. These features can be readily assigned by spectra to provide the deconvolution of the silicon atoms of silanol groups on the surface, which were described as the (Q2), (Q3), and (Q4) species, respectively [26,27].

**Growth inhibition of A. solani under laboratory conditions**

*A. solani* growth was significantly inhibited by MSN and metalaxyl at various concentrations compared with the untreated control. However, the highest growth inhibition percentage of *A. solani* was achieved at the highest concentration (400 mg/L) (Table 1 and Fig. 4). The degree of growth inhibition positively correlated with MSN and metalaxyl concentration level.

**Efficacy under greenhouse conditions**

MSN and metalaxyl significantly reduced the severity of early blight of tomato as compared with untreated control in two growing seasons (Table 2). The degree of severity reduction positively correlated with concentration levels of the tested materials. The severity reduction was higher in the second season than in the first one.

**Effects on growth parameters**

The effect of MSN on the growth characters of tomato plants was assessed by comparing with metalaxyl (Table 3). The measured growth parameters were plant height, fresh weight, and dry weight. The tomato growth characters were significantly increased in treated plants compared to the untreated control. Fresh and dry weight of tomato plants treated with MSN increased two fold more than untreated control in both growing seasons.

**Discussion**

Early blight is a fungal disease that caused by *Alternaria solani* that occurs on tomatoes worldwide. This fungal disease is generally one of the most severe tomato problems faced and if uncontrolled, early blight can cause significant yield reduction [28]. Therefore, for top yield of high quality tomato fruit, control of this
Effect of the MSN and metalaxyl treatments on the severity of *A. solani*. The physical characteristics and structural features of MSN such as the high surface area, unique structure, cylindrically-shaped and uniform pore sizes led to its high antifungal efficacy against *A. solani*. The unique features of MSN such as high surface area (SBET) of 489 m²/g and small pore size (Dp) of 10.7 expected to improve the significance of its surface morphology participating in the cellular interaction i.e., the number of active sites coming in contact with the cell walls which renders cytotoxic effect against early blight fungus. Also, it is well known that the smaller particles have larger surface area available for interaction and will give more antimicrobial effect than the larger particles.

Any management strategy for plant pathogens should not focus only on the effective control of the pathogens that attack the agricultural crops. It should also take in consideration its effect on growth and yield characters of crops. Significant increase in growth parameters of tomato plants treated with MSN were recorded and this is agreed with findings of [43], who reported that the application of control agents suppresses diseases incidence and can have positive effects on plant growth and yield. This increase may be attributed to the fact that MSN and metalaxyl could reduce the influence of early blight fungus on the green area in tomato leaves; thus, decreased the damaged green leaf area which finally led to increase the plant growth because the leaves can undergo more photosynthesis [44].

Also, one of the most important factors is the amount of MSN that could be used under field conditions and its suitability/applied significance of its surface morphology participating in the cellular interaction i.e., the number of active sites coming in contact with the cell walls which renders cytotoxic effect against early blight fungus. Also, it is well known that the smaller particles have larger surface area available for interaction and will give more antimicrobial effect than the larger particles.

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Table 3
Effect of the MSN and metalaxyl on some growth parameters of treated tomato plants in the last season.

| Treatments | Plant height (cm) | Fresh weight/plant (g) | Dry weight/plant (g) |
|------------|-------------------|------------------------|----------------------|
| MSN        | 96.67 ± 2.88c     | 67.57 ± 0.30c          | 20.07 ± 0.15c        |
| Metalaxyl  | 85.00 ± 2.80b     | 45.33 ± 0.10b          | 12.90 ± 0.15b        |
| Control    | 61.57 ± 5.00a     | 28.60 ± 0.10 a         | 4.97 ± 0.10a         |

Each value is mean of four replicates. Mean ± SE followed by same letter in column of each treatment are not significant different at p = 0.05 as determined by Tukey–Kramer HSD.

Conclusions
MSN effectively reduced the severity of early blight disease in tomato and improved tomato growth characters also. It is expected that the application of MSN nanoparticles at low concentrations will be eco-friendly and decrease farm management costs. The easy synthesis of MSN is a highly promising approach to designing and synthesizing other metal oxides with powerful antifungal activity; that can be used as a safe alternative to chemical fungicides to control tomato early blight.

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