Synthesis and Antifungal Evaluation of Magnetic Magnesium Oxide Nanoparticles Against Fusarium Oxysporum

Jia Le Wee,[a] Yen San Chan,[a,*] Ming Chiat Law[b]

[a]Department of Chemical and Energy Engineering, Faculty of Engineering and Science, Curtin University Malaysia, CDT 250, 98009 Miri, Sarawak Malaysia
[b]Department of Mechanical Engineering, Faculty of Engineering and Science, Curtin University Malaysia, CDT 250, 98009 Miri, Sarawak Malaysia

[*]chanyensan@curtin.edu.my

Abstract. Hybrid nanoparticles (NPs) have received much interest over the past decades because they have the potential to overcome the limits of single-component particles. This study proposes a hybrid magnetic magnesium oxide (m-MgO) NPs to combat the plant pathogenic fungus, *Fusarium oxysporum* (*F. oxysporum*). The m-MgO NPs were synthesized via ultrasonic mediated sol-gel method. UV-visible spectrometry confirms the successful formation of m-MgO NPs. In addition, the magnetic activity of m-MgO NPs was illustrated through a preliminary magnetic activity study. A disc diffusion assay was carried out to determine the effectiveness of m-MgO NPs to inhibit the growth of *F. oxysporum*. The results showed that the zone of inhibition was 7.58 ± 0.30 mm at 10 mg/mL, suggesting that the synthesized m-MgO NPs are an effective fungicide to inhibit the growth of *F. oxysporum*.

Keywords: Agricultural applications, antifungal, *fusarium oxysporum*, nanoparticles, hybrid magnetic magnesium oxide nanoparticles

1. Introduction

Food insecurity is a major issue worldwide [1]. This is because food crops are hard to maintain due to their low yield and vulnerability to various crop diseases. The f*usarium* spp. fungus is reported to be one of the biggest threats in Sarawak, Malaysia's agricultural sector, causing diseases in plants such as the black pepper plant [2], banana tree [3], and maize plant [4]. *Fusarium* infection on a plant may result in low yield. Currently, conventional fungicides are unable to effectively control the spread of *Fusarium* diseases and are environmentally harmful, causing soil and water contamination [5]. Therefore, an alternative fungicide should be developed to combat this issue.

Magnesium oxide nanoparticles (MgO NPs) are metal oxide nanoparticles that have recently received much attention due to their excellent chemical and physical properties such as eco-friendliness, strong mechanical strength, great optical transparency and stability, and high corrosion resistance. Recent studies have shown that MgO NPs exhibit excellent antimicrobial activity against microorganism such as against *P. aeruginosa*, *B. subtilis*, *E. coli*, *S. aureus* [6], *A. niger*, *A. oryzae*, *R. solanacearum*, *K. pneumoniae* and *X. oryzae*. Besides, MgO NPs are not ecologically harmful as they do not affect the survival of *Eisenia Andrei* earthworms in soil. In addition, MgO NPs can act as plant growth promoters or fertilizers. Moreover, MgO NPs can enhance the physical and mechanical properties of soil, including soil porosity, mean weight diameter of aggregates, water content, saturated hydraulic conductivity, and

reduction in penetration resistance. On the other hand, iron oxide NPs have similar properties as well. They possess excellent antimicrobial properties, plant growth promotion, as well as magnetic properties. Furthermore, the transport of iron oxide NPs in plant can be controlled by an external magnetic field, which is also known as site-targeted delivery capability. This provides more effective and efficient disease treatment, as a higher concentration of NPs can be transported to the infected spot in a shorter time. The combination of iron oxide NPs and MgO NPs is known as a hybrid NPs, which have the potential and ability to overcome the limits of single component particles [7]. This is strong evidence to prove that magnetic MgO NPs (m-MgO NPs) have the potential to be developed as an antimicrobial agent for plant disease management. Hence, this manuscript reports the evaluation of the antifungal effect of m-MgO NPs against *F. oxysporum*.

2. Methodologies

2.1. Synthesis of m-MgO NPs

The m-MgO NPs were fabricated using the ultrasonic mediated sol-gel method [8]. Briefly, the precursor (magnesium acetate tetrahydrate), gelling agent (oxalic acid dihydrate) and magnetic agent (iron (III) nitrate nonahydrate) were separately dissolved in ethanol for 1 hour at 300 RPM, with a molar ratio of 2:4:1. Then, the three solutions were mixed for 1 hour at 300 RPM to form a gel solution. The gel solution was dispersed by using an ultrasonicator at 20 kHz, 60% amplitude, and 0.6 cycles for 5 minutes. After that, an aging process was carried out by leaving the gel at room temperature for 12 hours. Next, the excess solvent and impurities were removed by drying the gel at 100 ℃ for 24 hours in an oven. Then, an agate pestle and mortar were used to grind the dried gel into fine powder. Lastly, the powder was calcinated at a heating rate of 5 ℃/minute until 500 ℃ for 2 hours to produce m-MgO NPs.

2.2. Characterization of m-MgO NPs

Ultraviolet-visible (UV-Vis) spectroscopy, particle size analysis and preliminary magnetic activity study were used for the characterization study.

2.3. Fungus culture and preparation

In the present study, the common disease-causing fungus *F. oxypsporum* was used. The fungus was cultured in petri dishes containing potato dextrose agar (PDA) at 32 ℃ for 10 days. The 10-day old *F. oxysporum* was then stored in a regrigerator at 4 ℃ for later use.

To perform the antifungal assay, a spore solution of *Fusarium* was required. To prepare the spore solution, a sterile Tween 80 solution was first prepared by mixing 10 mL of deionized water with 2 drops of Tween 80 and autoclaving the mixture at 121 ℃ and 15 psi for 20 minutes. The sterile Tween 80 solution was then cooled to room temperature inside a Class II Biohazard Safety Cabinet. Next, 2 mL of the Tween 80 solution was added to the 10-day-old *F. oxysporum*, and the solution and mycelia were mixed with an inoculum loop. The mixture was then filtered through a gauze, and the filtrate was collected as the Fusarium spore solution, which was stored in a refrigerator at 4 ℃ for later use.

2.4. Disc diffusion assay

The effectiveness of synthesized m-MgO NPs against *F. oxysporum* can be done by measuring the zone of inhibition (ZOI). Briefly, a 6 mm sterile blank disc was soak in distilled water and 10 mg/mL m-MgO NPs solution, respectively, to serve as the control and experimental set. After that, 150 μL of Fusarium spore solution was spread well on a fresh PDA, followed by placing the prepared blank discs on it. Then, the PDA plates were incubated at 32 ℃. The diameter of ZOI for each sample was measured after 24 hours of incubation [9].

3. Result and Discussion

3.1. Synthesis and characterization of m-MgO NPs

During the synthesis of m-MgO NPs, a brown-coloured powder was formed, indicating that the m-MgO NPs are a combination of Fe_2O_3 and MgO NPs. The m-MgO NPs were further characterized using UV-Vis spectroscopy, particle size analysis, and a preliminary magnetic activity study.

In the UV-Vis spectroscopy study, the maximum absorption wavelength (λ_{max}) of m-MgO NPs was determined by carrying out absorption analysis from 800 to 190 nm, with deionized water used as a reference blank [10]. The absorption peak of Fe_2O_3 NPs was reported as ~210 nm and 380 nm by Zangeneh Kamali, Alagarsamy [11], while the absorption peak for MgO NPs was reported in the range of 260 – 280 nm [12]. However, the λ_{max} of the synthesized m-MgO NPs was found to be ~200 nm and ~260 nm, as shown in Figure 1. The discrepancy in second peak (~260 nm) could be due to the colorimetry changes caused by the combination of MgO and Fe_2O_3 NPs.

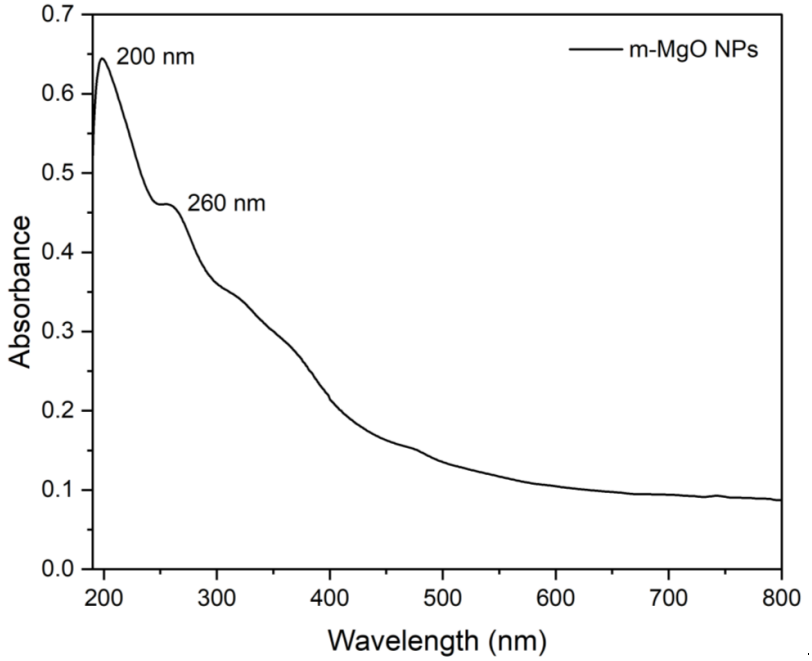

Figure 1 UV-Vis spectrum of m-MgO NPs

Besides, the average particle size of m-MgO NPs was determined using the dynamic light scattering (DLS) technique. The average particle size was found to be 308.40 nm, with a PDI value of 0.236. The larger size of the m-MgO NPs, which is greater than 100 nm, is likely due to the addition of magnetic agent during the synthesis process. During the hybrid NPs synthesis, the iron oxide may have formed a different shape than the MgO NP. For example, in a hydrothermal hybrid NPs synthesis, spherical MgO NPs were found to deposit on iron oxide nanorods [13]. In addition, the limitation of DLS may have contributed to the large particle size of the m-MgO NPs. DLS measurements assume that all particles are spherical in shape, thus particles with shapes other than spherical could have contributed to the variation in results [14].

Next, a preliminary study of the magnetic activity of m-MgO NPs was carried out using a magnet. The results demonstrated that m-MgO NPs exhibited excellent magnetic activity. Figure 2a depicts the

well-dispersed m-MgO NPs in deionized water. When an external magnetic field was applied, the m-MgO NPs were attracted toward the magnet, as shown in Figure 2b.

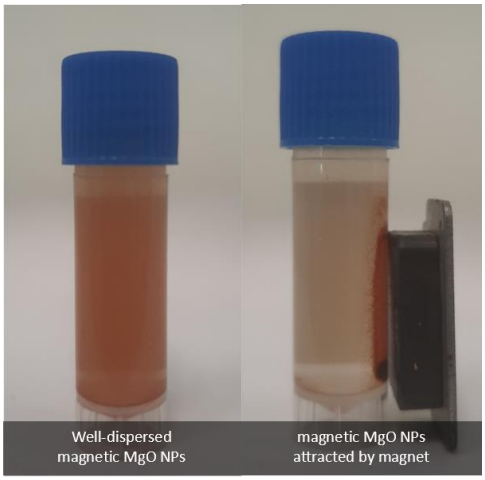

Figure 2 Demonstration of m-MgO NPs magnetic activity (a) without external magnetic fields (b) with external magnetic fields

3.2. Disc diffusion assay

The clear zone around the discs impregnated with m-MgO NPs indicates that they exhibit antifungal properties against the plant pathogenic fungus, *F. oxysporum*. The diameter of ZOI of distilled water and m-MgO NPs against *F. oxysporum* are measured, as shown in Figure 3. The average ZOI of m-MgO NPs was found to be 7.58 $\pm$ 0.30 mm, while no ZOI was observed in the distilled water samples. This indicates that the antifungal properties against *F. oxysporum* are due to m-MgO NPs. The release of Fe and Mg metal ions from the m-MgO NPs into fungus cell generates oxidative stress, leading to the inhibition of fungus growth [7]. Since both MgO and Fe_2O_3 NPs exhibit great antifungal activity, their combination, m-MgO NPs, is expected to demonstrate better antifungal activity. This is because hybrid NPs combine the characteristics of of both NPs/components into one [15].

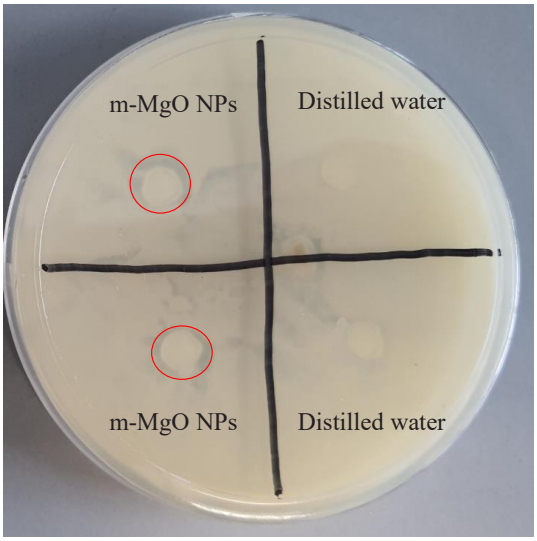

Figure 3 Zone of inhibition of m-MgO NPs and distilled water illustration

4. Conclusion

The m-MgO NPs have the potential to be developed as a fungicide to fight against the plant pathogenic fungus, *F. oxysporum*. However, further research is required before m-MgO NPs can be implemented in agricultural sector. For instance, a more detailed fungicidal activity study of m-MgO NPs, cytotoxicity of m-MgO NPs, impact of m-MgO NPs towards plant and environment, and accumulation of m-MgO NPs in soil.

5. Footnote

5.1. Competing interests

There are no conflicts to declare in this project.

5.2. Funding

This project is funded by Sarawak Research and Development Council (SRDC), project ID RDCRG/CAT/2019/05.

5.3. Acknowledgement

The authors would like to acknowledge SRDC for funding this project. Besides, the authors would like to appreciate the laboratory support by the engineering lab of Faculty of Engineering and Science, Curtin University Malaysia and Sarawak Biovalley Pilot Plant.

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