Magnesium Oxide Nanoparticles: A Dual-action Solution for Combating *Fusarium Oxysporum* and Enhancing Plant Growth

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***Abstract*—nano-Magnesium Oxide nanoparticles (n-MgO) have garnered significant attention in recent decades, particularly in the agricultural sector, due to their exceptional properties, such as antifungal potency and the ability to promote plant growth. In this study, the ultrasound-mediated sol-gel method was employed to synthesize n-MgO. Various physicochemical characterization techniques, including Ultraviolet-visible spectrometry, Fourier-transform infrared spectroscopy, and X-ray diffraction, were used to confirm the formation of n-MgO. Additionally, the study assessed the antifungal activity of n-MgO against the plant pathogenic fungus *Fusarium oxysporum* (*F. oxysporum*). The disc diffusion method was used to determine the effectiveness of n-MgO in inhibiting the growth of *F. oxysporum*. Subsequently, the Minimum Inhibitory Concentration (MIC) required to suppress the growth of *F. oxysporum* was determined through a broth microdilution study. Furthermore, a field study was conducted to evaluate the plant growth promotion capabilities of n-MgO. The results indicated an inhibition zone of 8.74 ± 0.16 mm, with an MIC of 2.5 mg/mL, suggesting that n-MgO effectively inhibit the growth of *F. oxysporum*. Moreover, the application of n-MgO in the field study resulted in a 5.78% increase in the height of maize plants. Consequently, it can be concluded that n-MgO holds significant potential as an effective agrochemical product with dual functions as a fungicide and a plant growth promoter.**

***Keywords*—agrochemical, antifungal activity, *Fusarium oxysporum*, magnesium oxide, nanoparticles, plant growth promotion**

I. Introduction

Agriculture plays a vital role as the primary source of food for people worldwide [1]. However, it faces multiple global challenges, including the prevalence of plant diseases caused by various pathogens in the environment (e.g., fungi, bacteria [2], nematodes [3], etc.), leading to significant losses in crop yields. Of particular concern are phytopathogenic fungi, especially Fusarium spp., which cause a range of diseases such as fusarium wilt and stalk rot in economically important crops, directly affecting global food security [4]. In addition, the impact of Fusarium is not limited to crop production losses, but may also trigger the production of mycotoxins, posing a significant threat to human health [5, 6]. Although various agrochemical products are available to farmers in the market, these products have proven to be ineffective against fusarium disease. Additionally, the use of these agrochemicals can lead to soil and water contamination that may harm plants [7]. Hence, there is a pressing need for a novel, innovative, and eco-friendly alternative for controlling fusarium diseases in agriculture.

II. Literature Review

Recently, various environmentally friendly and efficient alternatives such as plant extracts [8], essential oils [9], biological control [10], and engineered nanomaterials [11] have been widely studied for their possibility and capability to control phytopathogenic fungi. Among these alternatives, the uses of nanomaterials have been studied the most. Generally, engineered nanomaterials have different physiochemical properties compared to their bulk counterparts, which lead to better results when compared to conventional agrochemicals for plant disease control [12]. Up to date, different types of nanomaterials such as carbon nanomaterials [13], nanopolymers [14], and metal nanoparticles [11, 15, 16] have been studied as alternatives for controlling phytopathogenic fungi diseases. Metal oxide nanoparticles are considered to be an efficient and eco-friendly option for controlling phytopathogenic fungi diseases in the agricultural sector due to their structural stability, target affinity, high surface-to-volume ratio and nanoscale size [17]. Furthermore, the application of appropriate amounts of metal oxide nanoparticles helps increase seed germination and promote plant growth [18].

Nano Magnesium Oxide nanoparticles (n-MgO) is metal oxide nanoparticles that have received much attention recently. This is because of their excellent properties such as eco-friendliness [19], great optical transparency and stability, strong mechanical strength [20], and high corrosion resistance [21]. Recent studies have reported the antimicrobial activity of n-MgO, such as against *P. aeruginosa*, *B. subtilis* [22], *E. coli*, *S. aureus* [23], *A. niger* [24], *A. oryzae* [25], *R. solanacearum* [26], *K. pneumoniae* [27] and *X. oryzae* [28]. The n-MgO exhibits excellent antimicrobial activity due to its nanoscale size which could induce nanotoxicity to the microbes. For instance, the n-MgO can penetrate microbes due to its nanoscale size and cause cellular damage such as the formation and accumulation of reaction oxygen species as well as denature of ribosomes [29]. Besides, n-MgO is known to be environment friendly. For example, they do not affect the survival of *Eisenia Andrei* earthworm [30]. Furthermore, literature has pointed out the ability of n-MgO to act as a plant growth promoter or fertilizer [26, 31]. This is due to the fact that magnesium is one of the micronutrients that plant requires to support their fundamental function, including chlorophyll synthesis, photophosphorylation, photo-oxidation and many others [32]. Moreover, n-MgO can

enhance the soil's physical and mechanical properties, including soil porosity, saturated hydraulic conductivity, water content, mean weight diameter of aggregates, and reduction in penetration resistance [33]. These are the strong evidence to prove that n-MgO has the potential to be developed as an antimicrobial agent for plant disease management. Despite the appealing properties of n-MgO, the antifungal effects of n-MgO are scarcely reported. Furthermore, it is worth noting that the antimicrobial activity and plant growth promotion potential of n-MgO have typically been reported in separate studies, often involving n-MgO with varying sizes and shapes. These differences in the size and shape of nanomaterials can lead to distinct outcomes. Consequently, this manuscript aims to address this gap by presenting an integrated assessment of both the antifungal effects against *F. oxysporum* and the plant growth promotion capabilities of the same n-MgO.

III. Materials and Methods

A. Synthesis of n-MgO

The synthesis method of n-MgO was adapted from those described by Wong *et al.* [34] with slight modification. Briefly, equimolar of magnesium acetate tetrahydrate (precursor) and citric acid (gelling agent) were dissolved in ethanol separately for 1 h at 300 RPM. Then, both solutions were mixed by using magnetic stirrer at 300 RPM for 1 h. Formation of gel occurred during the mixing process. Next, the gel was ultrasonicated for 15 min. After that, the gel was aged at room temperature for 12 h to obtain a thicker gel. Next, the gel was dried in oven at 100 °C for 24 h to remove the excess solvent and impurity. The dried gel was then grounded with agate mortar and pestle to obtain fine powder. Lastly, the powder was calcinated in a box furnace at a heating rate of 5 °C/min until 650 °C for 2 h to produce n-MgO [35].

B. Characterization of n-MgO

Analytical techniques such as Ultraviolet-Visible (UV-Vis) spectroscopy, Fourier Transformed Infrared (FTIR) spectroscopy, and X-Ray Diffraction (XRD) were used for the characterization study.

C. Fungus Culture

Common disease-causing fungi, *Fusarium oxysporum* was used in this work. They were cultured in petri dishes that contained Potato Dextrose Agar (PDA) at 30 °C for 10 d. Then, the petri dishes were kept in 4 °C fridge for further use. Besides, *Fusarium* spore solution can be prepared with the procedure described below. 10 mL deionized water was added with 2 drops of Tween 80. This Tween 80 solution was then sterilized by using autoclave at 121 °C and 15 psi for 20 min. After that, the Tween 80 solution was cooled down to room temperature inside Class II Biohazard Safety Cabinet to prevent contamination. Next, 2 mL of the Tween 80 solution was added to the 10 d old *F. oxysporum*'s plate and mixed well by using inoculum loop. Then, the spore solution was obtained by filtering the mixture with gauze to remove the mycelium of fungi.

D. Antifungal Assay

1) Disc diffusion method

Disc diffusion method is a quick and inexpensive assay to test the antifungal activity of a compound by measuring the Zone of Inhibition (ZOI). Firstly, a 6 mm sterile blank disc was impregnated with various concentration (2.5, 5, and 10 mg/mL) of n-MgO solution, where another blank disc was impregnated with distilled water to serve as control. Then, 150 μL spore solution was spread on a fresh PDA. After that, the prepared blank discs were placed on the inoculated PDA. Next, agar plates were kept in incubator at 32 °C. The diameter of ZOI was measured after 24 h of incubation [36].

2) Minimum Inhibitory Concentration (MIC) determination

MIC indicates the lowest concentration of the test sample that inhibits the growth of microorganism. The MIC of n-MgO was determined by conducting the broth microdilution method on 96-wells plate with the aids of a microplate reader. Various concentrations of n-MgO (20, 17.5, 15, 12.5, 10, 7.5, 5, and 2.5 mg/mL) were used to test against *F. oxysporum* [37]. Initially, 40 μL of n-MgO solution with various concentrations were added into 8 rows of the 96-wells plate, accordingly, followed by 100 μL Potato Dextrose Broth (PDB). Next, 40 μL of $\times 10^6$ spore/mL of fungal spore suspension was inoculated to the 96-wells plate. Moreover, negative control (PDB + fungus) was prepared in the 96-wells plate as well for comparison purposes. After that, the 96-wells plate was incubated in the microplate reader for 24hrs, where the absorbance readings were taken on 0 h and 24 h. The Optical Density (OD) used was 600 nm, which is a common OD to monitor the fungus growth status. Then, the lowest n-MgO concentration sample that had a lower absorbance value than the positive control was served as MIC [37, 38]. This assay was conducted in six replicates to ensure the accuracy of the result.

E. Field Study for Plant Growth Promotion

A field study was carried out in a greenhouse to assess the plant growth promotion capability of n-MgO. Briefly, one-month-old maize seedlings were used for this field study. 10mL of n-MgO solution at its MIC were applied weekly until the maize plants reached the harvest stage. In parallel, a control group was grown under normal conditions. Thirty plants were included in each experiment to ensure the consistency of result. The height of maize plants was measured on a weekly basis throughout the course of the experiment as an indicator of growth.

IV. Result and Discussion

A. Confirmation of n-MgO Synthesis

During the synthesis of n-MgO, white powder formation (as shown in Fig. 1) after the calcination process confirms the synthesis of n-MgO [34]. Then, the n-MgO were further characterized by using UV-Vis, FTIR and XRD.

Fig. 1. Sample of n-MgO in powder form.

In the UV-Vis spectroscopy study, the maximum absorption wavelength (λ_{max}) of n-MgO was determined. This was done by carrying out the absorption analysis from 190 nm to 800 nm, while deionized water was used as a reference blank [39]. The absorption peak of n-MgO was reported in the range of 260–280 nm [40]. However, the λ_{max} of the synthesized n-MgO was found to be 224 nm, as shown in Fig. 2. This discrepancy could be due to the change in particle size or shape formation, as a result of the modified synthesis method.

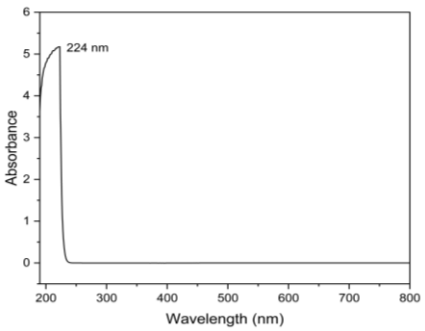

Fig. 2. UV-Vis spectrum of n-MgO.

On the other hand, Fig. 3 shows the FTIR spectrum of n-MgO, which include seven absorbance peaks at 3,699.3, 3,431.2, 1479.8, 1420.1, 859.1, 579.6, and 546.1 cm^{-1}. The absorbance peak at 3699.3 cm^{-1} is an impure/additional peak. The absorbance peak at 3431.2 cm^{-1} refers to the O-H stretch vibrational mode which shows the presence of alcohol [41]. Besides, the absorbance peaks at 1,479.8 and 1,420.1 cm^{-1} are responsible for the C-O-H bending and C-O stretch, respectively which confirms the presence of carboxylic acid. In addition, the absorbance peak at 859.1 cm^{-1} is due to formation of periclase MgO phase [42], while absorbance peaks at 579.6 and 546.1 cm^{-1} are due to MgO vibrations [35]. These three absorbance peaks confirm the formation of n-MgO. Moreover, the FTIR spectrums are similar as the one reported by Jeevanandam *et al.* [35], which further confirm the formation of n-MgO. However, slight shift in peak wavenumbers were identify. The modified n-MgO synthesis method is believed to be the reason that causes the change in shape formation, which in turn resulted in shifted peaks [43].

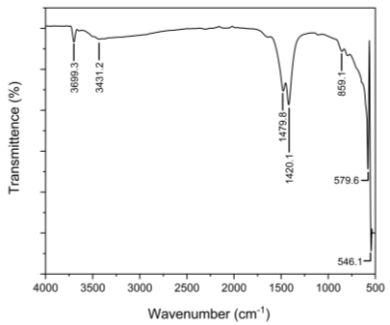

Fig. 3. FTIR spectrum of n-MgO.

B. Antifungal Assay

1) Disc diffusion method

The clear zone around the n-MgO impregnated discs indicates that n-MgO exhibit antifungal property against plant pathogenic fungus, *F. oxysproum*. The diameter of ZOI of various n-MgO concentrations against *F. oxysporum* are recorded in Table 1. Note that no ZOI was obtained in the case of control. This indicates that the antifungal property against *F. oxysporum* is due to n-MgO. Besides, the diameter of ZOI increases with increased n-MgO concentration. This means that the antifungal property of n-MgO against *F. oxysporum* is concentration dependent, where a higher n-MgO concentration results in a better fungus growth inhibition.

Table 1. Results of disc diffusion method

Sample	ZOI ± SD* (mm)
Deionized water	0.00
2.5	7.12 ± 0.06
5.0	7.63 ± 0.23
10.0	8.74 ± 0.16

*SD is standard deviation.

2) MIC determination

The results of microbroth dilution are summarized in Fig. 4. The absorbance value refers to the fungus density, where a higher absorbance value indicates a greater fungus density [44]. From Fig. 4, the negative control sample (0 mg/mL n-MgO) has the highest absorbance value, meanwhile all of the n-MgO treated samples have lower absorbance than the negative control. This indicates the n-MgO can inhibit the growth of *F. oxysporum*, which in turn resulted in lower fungus density. In current study. the MIC of n-MgO against *F. oxysporum* was reported to be 2.5 mg/mL. This is because it is the lowest concentration that have shown growth inhibition towards *F. oxysporum*. Besides, the absorbance values decrease with increased n-MgO concentration. This means that a higher n-MgO concentration can better inhibit the growth of *F. oxysporum*. This finding complies with the results found in disc diffusion method. Similar results were reported while using n-MgO as antimicrobial agent against pathogenic microbes [38, 45].

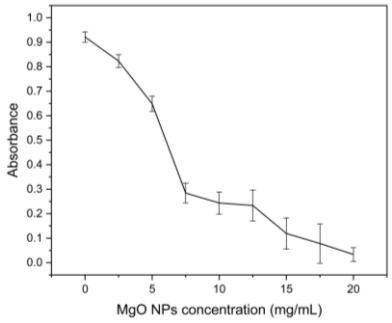

Fig. 4. Microbroth dilution assay of n-MgO against *F. oxysporum.*

C. Field Study

A field study was conducted to assess the impact of n-MgO on maize plants. Fig. 5 provides a summary of the maize plant heights over time. As depicted in Fig. 5, the maize plants treated with n-MgO displayed greater height compared to the control group at all time points, with the exception of

week 1. At the harvest stage, the n-MgO treated maize plant was 16.17 cm taller than the control group, representing a 5.78% increment. This is the Magnesium (Mg) element present in n-MgO is one of the essential micronutrients necessary for plant growth and development [46]. Magnesium plays a pivotal role in various physiological and biochemical processes crucial for plant health. These processes include facilitating energy metabolism by binding with ATP, promoting protein synthesis by stabilizing ribosomal association and activity, and supporting chlorophyll synthesis, which is vital for photosynthesis [11, 32]. This provides further evidence supporting the positive influence of n-MgO on enhancing maize plant growth. It is essential to note that plant height, while a significant metric, does not exclusively determine the overall growth status, as taller plants are not inherently superior to shorter ones. However, increased plant height can offer several potential advantages [47]. For instance, taller plants can more effectively intercept light and gain a competitive edge, leading to improved resource acquisition and utilization from the environment, ultimately promoting overall plant growth [48]. Significantly, the application of n-MgO in this field study did not yield any adverse effects but, rather, had the potential to enhance the growth of maize plants.

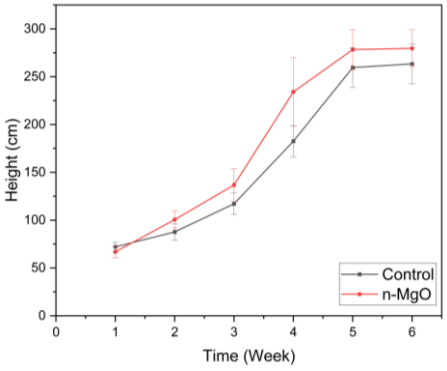

Fig. 5. Height of maize plant in field study.

V. Conclusion

This paper presents the successful synthesis of n-MgO using the ultrasound-mediated sol-gel method. The synthesized n-MgO was meticulously characterized using UV-Vis, FTIR, and XRD physicochemical characterization techniques. Additionally, this study evaluated the antifungal activity of n-MgO against *F. oxysporum* through the disc diffusion method, yielding a zone of inhibition measuring 8.74 ± 0.16 mm. Besides, the MIC of n-MgO against *F. oxysporum* was found to be 2.5 mg/mL, which is relatively low when compared to the ineffective conventional fungicide. Moreover, the field study demonstrated that the application of n-MgO led to increased vertical growth in maize plants (16.17 cm or 5.78%), which is advantageous for light competition and, subsequently, overall plant growth improvement. As a result, n-MgO can be considered as a dual-action agrochemical, effectively combating fusarium diseases caused by *F. oxysporum* while simultaneously promoting the growth of maize plants.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Jia Le Wee: Conceptualization, Investigation, Methodology, Validation, and Writing—Original draft preparation. Yen San Chan: Conceptualization, Methodology, Supervision, and Writing—Review and editing. Ming Chiat Law: Conceptualization, Supervision, and Writing—Review and editing. All authors had approved the final version.

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