Research Article

Large Scale Synthesis of Green Synthesized Zinc Oxide Nanoparticles from Banana Peel Extracts and Their Inhibitory Effects against Colletotrichum sp., Isolate KUFC 021, Causal Agent of Anthracnose on Dendrobium Orchid

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Fungicides have been extensively used to control fungal diseases that affect several crops including ornamental crops. However, concerns have arisen due to a development of fungicide resistance and increasing incidences of fungicide toxicity effects on nontarget organisms. As zinc oxide nanoparticles (ZnO NPs) have demonstrated effective antimicrobial activity, this study is therefore aimed at synthesizing ZnO NPs from banana peels using a green chemistry method in a large scale and determines their physical properties including their inhibitory effects against a plant pathogen fungus causing anthracnose in orchids, Colletotrichum sp. Results from X-ray diffraction and scanning electron microscope indicated that the synthesized ZnO NPs were obtained without other crystalline impurities, and they were spherical in shape with the average diameter of 256 ± 40 nm, respectively. The absorption peak was found to be centered at ~370 nm with the optical band gap value approximately 2.8 eV. Fourier transform infrared spectroscopy analysis confirmed the presence of several functional groups on synthesized ZnO NPs.

1. Introduction

Zinc oxide nanoparticles (ZnO NPs) have been shown to efficiently control microbial growth [1]. They are cheaper than silver nanoparticles with very high photocatalytic efficiency [2] and more biocompatible than other inorganic photocatalytic materials such as titanium dioxide [3]. Therefore, they are popularly employed as an active antimicrobial agent in food packaging [4], in the textile industry [5], paints [6], and plastics [7].

In recent decades, green synthesis of nanoparticles has gained extensive attention because it is environmentally friendly, reliable, and cost-effective and does not require high
pressure or high energy [8]. Principally, the green synthesis of nanoparticles can be achieved by addition of reducing agents and/or metal capping agents such as flavonoids, phenols, enzymes, and aldehydes [9, 10] present in plants or microorganisms, including animals [11]. Several studies have reported successful green synthesis of ZnO NPs with antibacterial activity from different bioresources, for example, plants [12–14], microbes [15], algae [16], and biowastes such as vegetable peel [17], orange fruit peel [18], banana peels [19], and durian rind [20]. In addition, biosynthesis of ZnO NPs has also been conducted on their antifungal activity. For example, spherical ZnO NPs synthesized by Aspergillus terreus were reported to strongly inhibit against Aspergillus niger (causing black mold disease on some fruits), Aspergillus fumigatus (causing aspergillosis in human), and Aspergillus aculeatus (a causal agent of plant disease) [21]. Furthermore, green synthesis of ZnO NPs using flower extract of Nyctanthes arbor-tristisshowed to have efficient antifungal activity toward Alternaria alternata (causing leaf spot in plants), Aspergillus niger, Botrytis cinerea (causing grey mould rot), Fusarium oxysporum (rotting agent in plants), and Penicillium expansum (causing blue mould) [22]. Subsequently, green synthesized ZnO NPs have been demonstrated as high-potential antifungal agents against plant pathogenic fungi.

Colletotrichum spp. is the most common causal agent of postharvest disease known as anthracnose. It widely infects several tropical fruits [23, 24], a wide range of plants and vegetables [25] including an ornamental crop such as orchids [26, 27]. Infection of Colletotrichum sp. on orchids such as C. gloeosporioides [28] or C. boninense [29] causes depressed circular leaf spots known as anthracnose spots or the necrotic lesions leading to plant defoliation and death in severe cases. Hence, orchids anthracnose by Colletotrichum spp. has caused significance in production losses [28, 30]. Although anthracnose is mostly controlled based on fungicide treatment, Colletotrichum spp. has been reported to have the ability to develop resistance to fungicides in some crops [31–33]. Therefore, it is crucial to find alternative management to control infection of the plant pathogenic fungi such as Colletotrichum sp.

This study is therefore aimed at synthesizing ZnO NPs by a green chemistry using crude extraction of banana peels (Musa sapientum) in a large scale synthesis. Physical properties of synthesized ZnO NPs were then characterized for their structures, morphology, and absorption spectrum by using X-ray diffraction (XRD), scanning electron microscope (SEM), and UV-visible spectrophotometer, respectively. Moreover, the surface chemistry of nanoparticles was also analyzed using the Fourier-transform infrared (FTIR) spectrometer. Furthermore, antifungal activity of synthesized ZnO NPs against Colletotrichum spp. that infected Dendrobium sp. (Sonia Earsakul) [34] was also determined both in the in vitro assay and greenhouse tests.

2. Materials and Methods

2.1. Preparation of Banana Peel Crude Extract. Peels of medium ripe banana (Musa sapientum) were prepared as previously described in Ruangtong et al. [19]. In this study, 400% (w/v) of crude extract was prepared by extracting 3,200 mg of grounded banana peels in 800 mL of distilled water at room temperature for 1 h under constant stirring (VELP Scientifica). Next, crude extracts were filtered twice using filter cloth and then stored at 4°C until further use.

2.2. A Large Scale Synthesis of ZnO NPs by a Green Chemistry Using Banana Peel Extract. Firstly, 800 mL of 2 M zinc acetate solution (Ajax Finechem) was prepared using deionized water. Then, it was mixed with 800 mL of 400% (w/v) of banana peel crude extract at 30°C under constant stirring using a magnetic stirrer (VELP Scientifica). After 1 h, the mixture was adjusted to pH 12 with 10 M of NaOH solution. Next, the precipitants were filtered using a Whatman filter paper, grade 4 (GE Healthcare), then dried in a hot oven (Kelvitrone®) at 80°C overnight. Finally, white powder was collected, washed several times with distilled water, and further heated in a hot oven at 80°C until dried. This green synthesis was independently performed three times. For each synthesis, mass of obtained powder was measured twice using a four-digit analytical balance (Thomas Scientific).

2.3. X-Ray Diffraction (XRD). X-ray diffraction patterns were recorded using an X-ray diffractometer (Bruker d8 Advance) using Cu K radiation of wavelength = 0.1541 nm in the scan range 2θ = 20–80°. Phase search was then compared using ZnO wurtzite JCPDS number 00036-1451 [35].

2.4. UV-VIS Spectroscopy. The optical absorption spectra of synthesized ZnO dispersed in water (about 500 μg/mL) were recorded using the UV-VIS spectrophotometer (SHIMADZU). The measurement spectrums ranged between 300 and 600 nm. Next, the optical band gap of ZnO was determined using the Tauc plot [36].

2.5. Scanning Electron Microscope (SEM). Prior to analysis, green synthesized ZnO nanoparticles were mounted on aluminum stubs and coated with gold film. Visualization of ZnO morphology was performed using a SEM (FEI). The size of particles was then analyzed using the ImageJ program [37].

2.6. Fourier Transform Infrared Spectroscopy (FTIR) Analysis. Grounded banana peels and synthesized ZnO NPs were analyzed using a Vertex 70, Platinum ATR (Bruker) by collecting spectra at room temperature under atmospheric pressure, at an average of 32 scans with a resolution of 4 cm⁻¹. The ATR mode was performed from 200 to 4,000 cm⁻¹. The IR spectrum table (Merck) was then used to determine functional groups and compound class.

2.7. Fungal Isolation. Symptomatic orchid plants, Dendrobium sp. (Sonia Earsakul), were collected in June 2020 to determine the causal agent. Fungal isolation from orchid leaves was carried out according to technique tissue transplanting described in Agrios (2005) [38]. In detail, infected leaves were cut into 0.5 cm × 0.5 cm, thoroughly cleaned and immersed in a solution containing 10% of NaOCl (The Clorox® Company, Jiangsu China) for 3 min. The leaves were rinsed with sterile distilled water (SDW) twice and dried on sterile filter paper. Next, the leaves were placed on a potato dextrose agar (PDA; Difco Oxford, UK) and incubated at 25 ± 2°C until the mycelium was developed on
the infected leaves. The mycelium was then subcultured to fresh PDA to obtain pure cultures for identification.

2.8. Identification and Characterization of Colletotrichum sp. Three isolates of Colletotrichum sp. including KUFC 021, KUFC 022, and KUFC 023 were obtained, the cultures were grown on PDA for 7 days at 25 ± 2°C. The macroscopic features of the fungi were observed, such as colony color, growth rate, fungal pigment production, and fruiting body. The microscopic characteristics such as acervuli and conidial features were examined under stereo (Olympus, Tokyo, Japan) and compound (Carl Zeiss, Jena, Germany) and compared with the features in identification keys and species descriptions [39].

2.9. Pathogenicity Test. Based on the sporulation, the Colletotrichum sp. isolate KUFC 021 was selected for pathogenic testing on three five-month-old dendrobium plants in a greenhouse. Five leaves wounded with needles were inoculated by spraying with a conidial suspension of 10^6 conidia/mL prepared from the isolate. Plants inoculated with SDW served as control. The inoculated plants were incubated in a moist chamber at 25 ± 2°C for 48 h and then maintained for 7 days in a greenhouse for symptom evaluation [26].

2.10. Antifungal Activity In Vitro Test of Green Synthesized ZnO NPs. In the antifungal activity of green synthesized ZnO NPs to control Colletotrichum sp., isolate KUFC 021 was tested in triplicate in vitro on PDA. Mycelial disks (5 mm in diameter removed from the margins of 7 days old cultures) were transferred to PDA amended with ZnO NPs at six different concentrations (5,000, 7,500, 12,500, 15,000, 17,500, and 20,000 mg/L). The experiment was conducted by including the negative control agar plate (no ZnO NPs) and two positive controls, which were PDA plates containing a contact fungicide (mancozeb 80% W/W WP, Corteva Ltd., Thailand) and a systemic fungicide (carbendazim 50% W/W WP, Erawan Ltd., Thailand). Five replicate plates of PDA per treatment were incubated at 25 ± 2°C. Antimicrobial activity was evaluated by measuring the colony diameter at 7 and 14 days after incubation. The percentage of mycelial growth inhibition was calculated as \( (A - B/A) \times 100, \) where \( A \) and \( B \) are the diameter of a fungal colony grown in a negative control plate and the diameter of a fungal colony grown in a plate containing synthesized ZnO NPs, respectively. Then, the EC_{50} values of ZnO NPs against Colletotrichum sp. (KUFC 021) were calculated using GraphPad QuickCalcs (GraphPad software).

2.11. Greenhouse Evaluation of Antifungal Activity from Green Synthesized ZnO NPs. The effectiveness of green synthesized ZnO NPs was evaluated for the control of anthracnose disease of orchids in vivo. One-hundred and twenty of Dendrobium sp. (Sonia Earsakul) were planted in the greenhouse and arranged in a completely randomized design (CRD) for six treatments (positive control, negative control, 1 g/L of carbendazim, 1.5 g/L of mancozeb, 20 g/L of ZnO NPs, and 30 g/L of ZnO NPs) with twenty replications per treatment. Five treatments were sprayed with a spore suspension of Colletotrichum sp. (KUFC 021) at 10^8 conidia/mL while the negative control was sprayed with water. Plants were then covered with plastic bags for 24 hours. The plastic bags were removed after inoculation, and two different concentrations of green synthesized ZnO NPs at 20 and 30 g/L were applied to orchid plants and compared to 1 g/L carbendazim and 1.5 g/L mancozeb utilizing foliar sprays. For positive control, the plants were sprayed with water. The percentage of disease severity index and level of disease [40] was calculated at 7 and 14 days after application. Disease severity was scored on a 1 to 5 scale, where level 1 indicates no infection, healthy; level 2 indicates infection 1-10%, and the leaves area shows necrotic lesions; level 3 indicates infection 11-20%, and the leaves area shows dark brown lesions with acervuli; level 4 indicates infection 21-50%, and the leaves area shows dark brown lesions/lesions that coalesce to form enlarged legion, and level 5 indicates infection greater than 50%, heavily infected leaf turning completely brown and forming several concentric rings.

2.12. Statistical Analysis. Data were analyzed using SPSS (version 22) statistical software. The effect of different concentrations of synthesized ZnO NPs on the growth of fungi was evaluated by one-way analysis of variance (ANOVA). Duncan’s multiple range test was used to compare the differences among treatments. \( P \) values less than 0.05 were considered statistically significant.

3. Results

3.1. Physical Characteristics of Green Synthesized ZnO NPs from Banana Peel Extract in a Large Scale Synthesis. This study was designed to simplify a green synthesis of ZnO NPs without requirement of a laboratory centrifuge or microwave and aimed to obtain high yield of ZnO NPs. By conducting three independent syntheses, a white powder was obtained in each synthesis with the average amount of 177.25 ± 8.17 g. All three syntheses offered high crystallinity of ZnO and zincite (JCPDS No. 00-036-1451) without other crystalline impurities as demonstrated in a representative XRD spectrum (Figure 1(a)). An illustrative SEM image shows that most of the synthesized ZnO were spherical and short oval shapes with the average diameter of 256 ± 40 nm (Figure 1(b)). The optical property of the synthesized ZnO NPs was then studied using UV-visible spectroscopy. As a result, the dispersed ZnO NPs in deionized water shows the absorption peak centered at ~370 nm (Figure 1(c)). By using the Tauc plot [36], the calculated optical band gap value was found to be ~2.8 eV. In addition, the FTIR spectrum indicates the presence of several functional groups on the green synthesized ZnO NPs (Figure 1(d)). The characteristic peaks obtained between 600 and 450 cm\(^{-1}\) confirms the Zn-O stretching bonds, whereas the peak at 3427 cm\(^{-1}\) belongs to the O-H stretch of the carboxylic acid group that was found in both ZnO NPs and banana peels. Moreover, the peaks at 1638 cm\(^{-1}\), 1536 cm\(^{-1}\), and 1407 cm\(^{-1}\) of synthesized ZnO NPs correspond to C=O stretching, N-O stretching, and O-H bending, respectively (Figure 1(d)).
3.2. Characterization of Colletotrichum sp. Isolates Causing Anthracnose of Orchids. Three isolates of fungi including KUFC 021, KUFC 022, and KUFC 023 were obtained from infected leaves of *Dendrobium* sp. (Sonia Earsakul) with anthracnose symptoms such as numerous black blemishes across the leaf and leaf tips turning brown (Figure 2(a)). Subsequently, both cultural and morphological characteristics were examined. After 7 days, the colony color of the isolates was taupe with average diameter of 8.5–9 cm (Figure 2(b)). Cylindrical conidia were produced inside the black acervuli that was filled with orange spore masses (Figure 2(c)). Setae was also produced and found to be acicular with dark brown color (Figure 2(d)) while appressoria were ovoid to slightly irregular in shape and had dark brown color (Figure 2(e)). Spores had rod-shape and colorless ranging from 12–17 × 3–6 μm in size (Figure 2(f)). Taken all together, morphological characterization suggested that all isolates were *Colletotrichum* sp. To confirm *Colletotrichum* species, molecular characterization is required for a future experiment.

In addition, a pathogenicity test was performed. Dark brown lesions developed on margin of leaves. On the lesion, salmon-colored conidial masses formed concentrically, which resembled symptoms that occurred in the field, were observed on leaves at 7 to 10 days after inoculation, while these symptoms did not occur in the control plants (data not shown). The same fungus was reisolated from the inoculated plants. Pathogenicity test revealed that the isolates of *Colletotrichum* sp. (KUFC 021) were pathogenic to *Dendrobium* sp. (Sonia Earsakul), thus satisfying Koch’s postulates.

3.3. A Large Scale Synthesis of ZnO NPs from Banana Peels Possessed Antifungal Activity against Colletotrichum sp. (KUFC 021) In Vitro. To study antifungal activity of green synthesized ZnO NPs against *Colletotrichum* sp. (KUFC 021), inhibitory effects of different concentrations of dispersed ZnO NPs in deionized water on the fungal growth were analyzed in different time points. From Figure 3, the radial growth of *Colletotrichum* sp. (KUFC 021) was significantly demolished by 1,000 mg/mL (manufacturer-recommended dose) of the commercial systemic fungicide and carbendazim in all time points. In contrast, 1,000 mg/mL of both mancozeb (the commercial contact fungicide) and the synthesized ZnO NPs could not inhibit the fungal growth at any time points. However, 4,000 mg/mL of ZnO...
NPs moderately inhibited growth of *Colletotrichum* sp. (KUFC 021) on day 7, and the effects were slightly reduced on day 10 and day 14. Next, the antifungal activity assay was further conducted using higher concentrations of ZnO NPs to determine the effective control to 50% growth inhibition (EC$_{50}$) against the growth of *Colletotrichum* sp. isolate KUFC 021. As a result, the antifungal activity of the synthesized ZnO NPs was exhibited in a dose-dependent manner. The best inhibitory effect was observed on day 7 with the EC$_{50}$ value equaling 13,991.6 mg/mL (Figure 4). Then, their effect was found to be slightly decreased on day 9 (EC$_{50}$ values = 14215.4 mg/mL). On the other hand, the least inhibition was shown on day 3 (EC$_{50}$ = 17,717.5 mg/mL). Therefore, the results implied the optimal concentration and time for treatment of ZnO NPs against *Colletotrichum* sp. (KUFC 021).

3.4. Effects of Synthesized ZnO NPs from Banana Peels on Growth of *Colletotrichum* sp. (KUFC 021) In Vivo. Next, antifungal activity of synthesized ZnO NPs was evaluated in vivo. From Figure 5, the typical symptom of anthracnose was clearly developed on leaves of the positive control (+control) that were inoculated with the *Colletotrichum* sp. (KUFC 021) suspension. The greenhouse condition, treated orchid plants with synthesized
ZnO NPs showed less disease symptoms on their leaves as same as carbendazim and mancozeb.

Calculation of the level of disease and percentage of disease severity index confirmed that the treatment of 30 g/L of the synthesized ZnO NPs were comparable with the recommended dose of the manufacturer for carbendazim (1 g/L) on day 7 and significantly better on day 14 (Table 1). Moreover, effects of synthesized ZnO NPs were also equivalent to 1.5 g/L of mancozeb (Table 1).

4. Discussions

Synthesis conditions of ZnO NPs have been reported to significantly affect sizes and shapes of particles that in turn influence their physical and biological properties. The crucial factors include types [41] and concentrations of precursors [19], temperatures [42], and procedure [12]. For the green synthesis, biological entities and their concentrations [43] are also important factors that affect morphology of ZnO NPs. Previously, we synthesized ZnO NPs from banana peel extract and obtained an average yield of a laboratory scale (0.5-1.0 g) [19]. In this study, we simplified the synthesis procedure by using a filter paper instead of a centrifugation and increased volume of both percussor and banana peel extract. Herein, we obtained approximately a 170-fold increase in the yield of ZnO NPs without the presence of other crystalline contaminations. Since this study can produce an average of 177 g per synthesis reaction, this synthesis condition is viable for providing the effective dose for controlling the disease in greenhouses. With the quality control check on the shape and size of ZnO NPs, the production cost was around $0.55 USD. This price is comparable to that of commercial ZnO NPs on the market. Moreover, the physical properties of newly synthesized ZnO NPs including the photoluminescence spectrum and optical bandgap were in accordance with other green synthesized ZnO NPs using different biological extracts [44, 45]. Although the FTIR result also confirmed the functional groups of phytochemicals presented in the banana peel extract as reported previously [19], a modified procedure for this synthesis resulted in the new morphology of ZnO NPs with round shape about 256 ± 40 nm.

Numerous studies have shown that green synthesized ZnO NPs possess potent antibacterial activity while few studies have addressed their antifungal effects. This study firstly evaluated antifungal activity of green synthesized ZnO NPs from banana peel extracts against Colletotrichum sp. Our synthesized ZnO NPs significantly inhibited growth of Colletotrichum sp. (KUFC 021) and drastically reduced anthracnose symptoms. Despite their inhibitory effects being weaker than that of carbendazim and mancozeb, prolonged inhibitory effects of the synthesized ZnO NPs against Colletotrichum sp. (KUFC 021) were observed in the greenhouse, and it tended to be better than both mancozeb and carbendazim. In addition, toxicity of both carbendazim and mancozeb has raised a serious concern on safety. For example, carbendazim has been reported to induce testicular toxicity and immature spermatids in male rats [46] and even its low dose (10 mM or 1.91 g/L) could significantly affect liver and hematole of the mouse model [47]. Similarly, mancozeb has been shown to cause oxidative stress in mammalian cell lines, thyroid disease [48], acute neurotoxic effect, and mitochondrial dysfunction in adult experimental animals [49]. Notably, carbendazim and mancozeb are fungicides that are heavily used to control fungal disease in orchids [50]. Therefore, a development of safe alternative fungicide is crucial. De la Rosa-Garcia et al. (2018) showed that chemical synthesized ZnO NPs (size ~26-37 nm) by coprecipitation and hydrothermal methods significantly inhibited growth of isolated C. gloeosporioides from avocado with the minimum inhibitory concentration equals to 0.312 mg/mL on PDA plates [51]. Moreover, Pariona et al. (2020) reported that 1 mg/mL of chemical synthesized ZnO NPs with platelet shape (size ~ 246 ± 40 nm) using a hydrothermal method suppressed growth of C. gloeosporioides on PDA plates by 60% inhibition that was stronger than ZnO NPs with rod (size ~ 780 × 142 nm) and spherical (size ~ 18 ± 2 nm) shapes [52].

In addition, foliar application of ZnO NPs has been reported to enhance plant growth, fruit yield, and biomass accumulation in various plants such as habanero pepper plants [53], maize [54], wheat [55], and foxtail millet [56]. However, depending on the plant size, plant type, NP concentrations, exposure time, and plant species, NPs might produce phytotoxicity, cytotoxicity, genotoxicity, or oxidative stress in plants [57, 58]. Although the orchid plants treated with ZnO NPs appeared to be healthy throughout our experiment, a long-term investigation is needed to determine the impact of green produced ZnO NPs on orchid plant growth.

![Figure 3: Inhibitory effects of synthesized ZnO NPs against growth of Colletotrichum sp. (KUFC 021) on PDA plates.](image-url)
Taken together, appropriate morphology of ZnO NPs may serve as a novel antifungal agent. We are aware that our green synthesized ZnO NPs require high doses to abolish growth of *Colletotrichum* sp. We are currently carrying out experiments by using different biomaterials for green synthesis.

**Figure 4:** Antifungal activity of different concentrations of synthesized ZnO NPs against growth of *Colletotrichum* sp. (KUFC 021). (a) Inhibitory effects on PDA plates at different time points. (b) Quantitative analysis and EC<sub>50</sub> values of synthesized ZnO NPs.

**Table 1:** Effects of synthesized ZnO NPs against *Colletotrichum* sp. (KUFC 021) in a greenhouse. Lower case letters represent significant differences determined by ANOVA, followed by Duncan’s posthoc test.

| Treatment | Day 7 | Day 14 |
|-----------|-------|--------|
| ZnO NPs (20 g/L) | 2.0<sup>b</sup> | 2.10<sup>b</sup> |
| ZnO NPs (30 g/L) | 1.70<sup>b</sup> | 1.80<sup>ab</sup> |
| Carbendazim (1 g/L) | 1.75<sup>b</sup> | 2.10<sup>b</sup> |
| Mancozeb (1.5 g/L) | 1.70<sup>b</sup> | 1.90<sup>ab</sup> |
| Control (+) | 3.20<sup>c</sup> | 4.30<sup>c</sup> |
| Control (-) | 1.10<sup>a</sup> | 1.55<sup>b</sup> |

Taken together, appropriate morphology of ZnO NPs may serve as a novel antifungal agent. We are aware that our green synthesized ZnO NPs require high doses to abolish growth of *Colletotrichum* sp. We are currently carrying out experiments by using different biomaterials for green synthesis.
synthesis with combination of metal doping for improving physical and biological properties of synthesized ZnO NPs.

5. Conclusion

This study highlighted a large scale synthesis of ZnO NPs from banana peel extracts using a green synthesis. The synthesized ZnO NPs with round shape, size ~ 256 ± 40 nm, were obtained without other crystalline impurities and had average energy band gaps ~2.8 eV. High doses of the synthesized ZnO NPs significantly suppressed growth of isolated Colletotrichum sp. (KUFC 021) from orchid plants on the culture plates. Moreover, they drastically reduced anthracnose symptoms on inoculated leaves with Colletotrichum sp. (KUFC 021) in the greenhouse condition.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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