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Synthesis of TiO_2-Ag composite through ultrasonic batch cleaning technique as a candidate for antifungal agent *Phytophthora palmivora*

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Abstract. In this work, TiO_2-Ag composites have been synthesized through the Ultrasonic Batch Cleaning technique and applied as an antifungal candidate against *Phytophthora palmivora*. *P. palmivora* is a pod rot fungus on cacao plants. Physically, the successful synthesis of TiO_2-Ag has been characterized by a change in the color of the precipitate from white to grey. This success was confirmed by Scanning Electron Microscopy (SEM) analysis, where the presence of Ag^+ ions made the TiO_2 surface more regular. The Ag^+ ion was evenly distributed on the TiO_2 surface and covers the large TiO_2 particles. The results of EDX analysis have shown that the % composition of TiO_2-Ag was 93.22%, each consisting of Ti of 50.37%, O of 37.57%, and Ag of 5.25%. Based on the antifungal activity test, TiO_2-Ag has good antifungal activity against *P. palmivora*. The minimum inhibitory level occurred at a concentration of 2.50%, while the minimum lethal rate occurred at a concentration of 4.88%.

1. Introduction

P. palmivora is a pathogenic fungus that causes pod rot in cocoa. This fungal attack occurs globally on cocoa pods and has an impact on decreasing crop productivity of cocoa beans in countries such as the Ivory Coast, Ghana, Nigeria, Cameroon, Brazil, Ecuador, Malaysia, and Indonesia [1]. Diah Lia Aulifa et. al. [2]. Antifungal *Phytophthora Palmivora* From Clove Buds (*Syzygium Aromaticum L.*) Nor Amerulah et al. [3] Antifungal efficacy of crude aqueous weed extracts against pathogens of cocoa black pod rot. Spraying chemical pesticides regularly has become the main choice of farmers in overcoming this problem, and its use is reported to be increasing every year.

Currently, the use of chemical pesticides has not shown optimal performance. Apart from being caused by the unstable nature of the compound, pesticide performance is not optimal due to its resistance effect [4]. Another challenge with the use of chemical pesticides is that chemical pesticides are pollutants for heavy metals on cocoa plantation soils [5]. So that its excessive use will cause new problems to the environment. Several strategies have been carried out as an effort to replace the role of chemical pesticides in overcoming *P. palmivora*, such as the development of natural pesticides [6,7] but has not shown significant results. Thus, the development of *P. palmivora* antifungal agents has become an interesting concern in recent years.

In this work, we report the performance of antifungal agents based on Ag^+ ion-modified TiO_2 semiconductor materials (TiO_2-Ag) against *P. palmivora*. The development of TiO_2 as an antifungal *P. palmivora* is an interesting study due to the nature of TiO_2 which is environmentally friendly, does not cause resistant effects, and is economical [8,9]. In general, testing the antifungal activity of TiO_2 against several fungi has been reported, such as *Helminthosporium maydis, Aspergillus niger, Fusarium graminearum, and Fusarium oxysporum, Hypocrea lixii, and Mucor circinelloides* [8–13]. For a wider application as an antifungal, TiO_2 can be modified through doping using both metal and non-metal compounds. This modification has shown a good increase in antifungal activity [14–16]. The choice of Ag^+ ion as a modifier of TiO_2 in this work is because, in the last years, Ag^+ ions have been widely used and developed as an anti-bacterial and antifungal agent. Based on several relevant studies, Ag^+ showed excellent performance on several fungi such as *Penicillium, Aspergillus, Clodosporium, Stachybotrys, Chaetomium, Candida albicans*, and *Saccharomyces cerevisiae*. Ag^+ is also reported to have high toxicity to microorganisms [17,18].

2. Materials and methods

2.1 Materials

The materials used in this work were DI Water, titanium isopropoxide (TTIP, 97%, Sigma-Aldrich), ethanol 99% (Sigma-Aldrich), silver nitrate ($AgNO_3$, Sigma-Aldrich), Potato Dextrose Agar (Sigma-Aldrich), the fungicide dithane M-45, and the fungus *P. palmivora* obtained from the Faculty of Agriculture, University of Halu Oleo.

2.2 Synthesis of TiO_2-Ag composites

A total of 10.0 mL of Titanium isopropoxide (TTIP) was added slowly to the Erlenmeyer flask containing 25.0 mL of ethanol, followed by the addition of 0.050 g of $AgNO_3$. The mixed solution was homogenized, then sonified for 60 minutes using an ultrasonic cleaning batch at a frequency of 40 kHz and an ultrasonic intensity of 0.96 kWm^{-2}. The precipitate formed is evaporated at 100°C for 2 hours and calcined at 500°C for 2 hours.

2.3 Preliminary test of TiO_2-Ag activity against P. palmivora

The preliminary test of TiO_2-Ag activity as an antifungal candidate of *P. palmivora* was carried out by the solid dilution method. In summary, the antifungal agent TiO_2-Ag with mass variations, 0.005 g, 0.015 g, 0.025 g, and 0.050 g, respectively, were added to the Eppendorf tube, then added 15 mL of PDA media. The PDA- (TiO_2-Ag) mixture was poured into a petri dish and allowed to stand until solidified. Furthermore, *P. palmivora* mushrooms that have been rejuvenated are cut using inoculation needles and planted in the middle of PDA- (TiO_2-Ag) media. The incubation process was carried out at room temperature, while the observation of the antifungal activity of TiO_2 doped Ag was carried out from the first day of incubation to the 30th day.

3. Results and discussion

3.1 SEM-EDX characterization

Figure 1a shows the reaction mechanism in the formation of TiO_2-Ag composites. The formation of TiO_2-Ag occurred in 3 important stages, namely the hydrolysis of TTIP, condensation of Ti $(OH)_4$, and the formation of TiO_2-Ag composites. Equation (i) shown that the hydrolysis of TTIP by H_2O molecules produces Ti $(OH)_4$. Furthermore, Ti $(OH)_4$ undergoes condensation to produce TiO_2. The presence of Ag^+ ions in the reaction system will then bind with the TiO_2 molecule to form a TiO_2-Ag composite as shown in equation (ii). Physically, the success of the synthesis process was characterized by a change in the color of the precipitate as shown in Figure 1b. The Ag^+ ion caused the change in TiO_2 color from white to gray.

Based on SEM analysis (Figure 2), there was a very significant difference between the surface morphology of TiO_2 (Figure 2a) and TiO_2-Ag (Figure 2b). TiO_2 was composed of various particle sizes and has an uneven surface shape. The presence of Ag^+ ions caused the surface of the TiO_2 to become more regular. The Ag^+ was evenly distributed on the TiO_2 surface and covers the large TiO_2 particles. Also, Figure 2 generally shown that the Ag^+ ion has a smaller particle size compared to TiO_2. The percentage composition (%) of the constituent elements of TiO_2-Ag composites was obtained through EDX analysis (Figure 2 insert). The results of this analysis indicate that there was an increase in the percent composition (%) due to the presence of Ag^+ ions. % composition of TiO_2 was 91.88%, meanwhile, TiO_2-Ag was 93.22% with a percentage value of each element, namely Ti of 50.37%, O of 37.57%, and Ag of 5.25%.

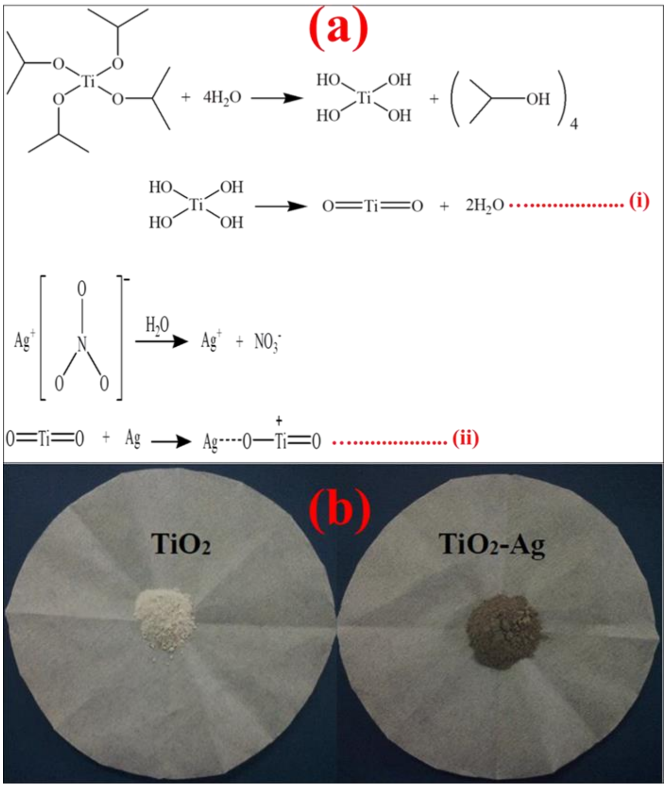

Figure 1. (a) The reaction mechanism for the formation of TiO_2-Ag composites; (b) Physical differences between TiO_2 and TiO_2-Ag

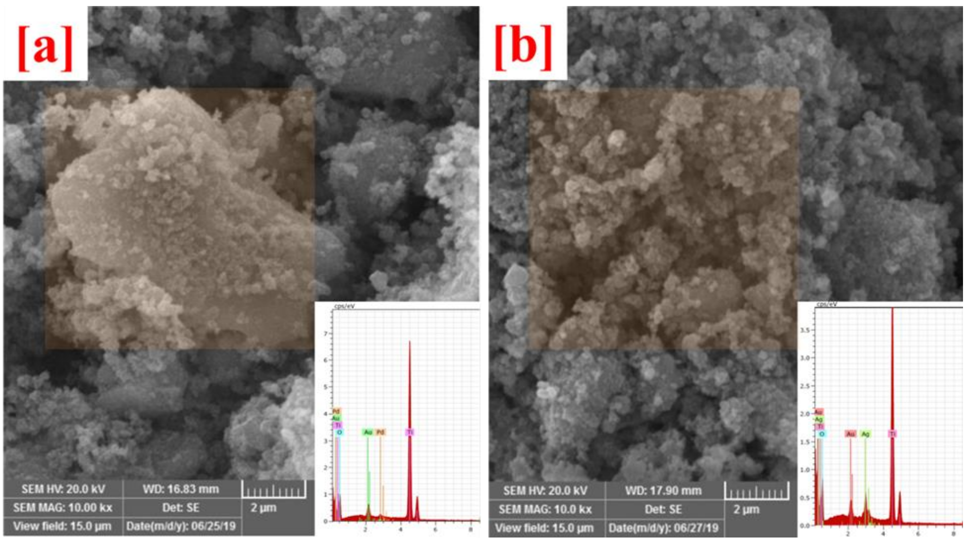

Figure 2. SEM-EDX: (a) TiO_2; (b) TiO_2-Ag

3.2 TiO_2-Ag activity as an antifungal activity of *P. palmivora*

In this initial study, the activity of TiO_2-Ag against *P. palmivora* was carried out in dark conditions (no exposure). The TiO_2-Ag concentrations used were 0.85%, 2.50%, 4.88%, and 7.87%. As a comparison, a fungicide with the active ingredient dithane M-45 was used. The ability of TiO_2-Ag against *P. palmivora* was determined by comparing the growth of *P. palmivora* colonies growing on control media with media that had been added with TiO_2-Ag. The results of measurements of *P. palmivora* growth inhibition are shown in table 1.

Table 1. Measurement results of *P. palmivora* growth inhibition

Concentration of TiO_2-Ag (%)	Inhibition diameter (cm)			Average of inhibiton diameter (cm)	Inhibition (%)
	1	2	3		
0.85	1.50	1.40	1.50	1.50	40
2.50	0.90	0.90	1.00	0.90	60
4.88	0.00	0.00	0.00	0	100
7.87	0.00	0.00	0.00	0	100
Control	2.50	2.40	2.50	2.50	0
Dithane M-45 (4.88%)	0.00	0.00	0.00	0.00	100

Table 1 shows that the inhibition of *P. palmivora* by TiO_2-Ag was strongly influenced by concentration. In this case, it appears that the higher the TiO_2-Ag concentration, the higher the inhibitory activity. The 4.88% concentration of TiO_2-Ag had the same% inhibition as dithane M-45 4.88%. This concentration not only inhibited the growth but also effective in killing *P. palmivora*. In addition to% inhibition, based on table 1 it can be seen that the minimum inhibitory level of TiO_2-Ag occurs at a concentration of 2.50%, the minimum lethal rate occurs at a concentration of 4.88%.

4. Conclusion

The development of antifungal materials based on TiO_2-Ag composites has been successfully synthesized and studied for its activity against *P. palmivora*. The synthesis process using the Ultrasonic Batch Cleaning technique produces TiO_2-Ag composites with regular surface morphology and uniform particle size. The TiO_2-Ag test as a candidate for antifungal material showed good activity with a minimum inhibitory level and a minimum lethal rate produced, respectively, 2.50% and 4.88%.

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