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Antibacterial Activity and Preservation Application of Tea Essential Oil Microemulsion

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Abstract: *This study explores the antibacterial activity and preservation application of tea essential oil microemulsion. The results showed that on the fifth day of treatment, the 30% microemulsion achieved an inhibition rate of 77.9% against the mycelial growth of Colletotrichum musae, which was similar to the inhibition rate of 0.020 mg/L prochloraz. The 50% microemulsion inhibited the spore germination of Colletotrichum musae by 77.0%, comparable to the 0.5 mg/L prochloraz treatment. In vivo antibacterial activity experiments indicated that both tea essential oil microemulsion and prochloraz exhibited protective and therapeutic effects against Colletotrichum musae. The 100% microemulsion showed the highest disease control efficacy, similar to that of 250 mg/L prochloraz, achieving a disease control rate of over 88% against Colletotrichum musae.*

Keywords: *Tea; Essential Oil; Microemulsion; Banana*

1. Introduction

Banana is an important tropical fruit in China. Diseases such as anthracnose (Colletotrichum musae) shorten its shelf life, causing significant losses to the industry. Traditional chemical preservation methods often lead to pesticide residues and food safety concerns, highlighting the urgent need for green, economical, and safe preservation technologies. According to Fu Gang et al. (2009), the incidence of anthracnose in untreated ripe bananas can reach 90%, resulting in severe economic losses.In recent years, microemulsions have been widely used in the encapsulation of plant essential oils and the storage and preservation of fruits and vegetables due to their excellent wettability, permeability, and stability. Essential oil microemulsions perform better than essential oils alone. In an oil-in-water (O/W) microemulsion system, hydrophobic active components are encapsulated within micelles and protected by surfactants and co-surfactants, reducing volatility and enhancing stability (Zhang et al., 2014).Tea is an important medicinal and edible plant in tropical and subtropical regions with high development potential. Recent studies have demonstrated the strong antibacterial and antioxidant activities of tea extracts, particularly volatile oils. However, plant essential oils have disadvantages such as poor water solubility, high volatility, and sensitivity to light, heat, and oxygen. Microemulsion technology can effectively address these issues. This study aims to further investigate the inhibitory effect of microemulsion technology on Colletotrichum musae. Therefore, Colletotrichum musae was selected as the research subject to study the antibacterial activity of tea essential oil microemulsion.

2. Materials and Methods

2.1 Materials and Reagents

Test materials: Tea essential oil was purchased from Jiangxi Hailin Fragrance Co., Ltd. The standard freeze-dried strain of Colletotrichum musae (ACCC31244) was obtained from Shanghai Ailei Biotechnology Co., Ltd. The banana variety used was Brazilian banana, sourced from Nanbin Farm, Yazhou District, Sanya City, Hainan Province. The selected bananas were complete in comb shape, well-developed, at maturity stage 7–8, with a bright surface, free from pest and disease infections, mechanical damage, and uniform in size and shape. The bananas were washed, the residual floral organs were removed, and they were cut into individual fingers. The 25% prochloraz aqueous solution was purchased from Hainan Zhengye Zhongnong High-Tech Co., Ltd.

Test reagents: Tween 80, absolute ethanol, potato dextrose agar (PDA) (Beijing Luqiao Technology

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Co., Ltd.), and potato dextrose broth (PDB) (Beijing Solarbio Science & Technology Co., Ltd.).

Culture media: PDA solid medium was prepared by dissolving 4 g of PDA in 100 g of distilled water, followed by autoclaving at 121°C for 20 minutes. PDB liquid medium was prepared by dissolving 3.5 g of PDB in 100 g of distilled water, followed by autoclaving at 121°C for 20 minutes.

2.2 Instruments and Equipment

BSA224S analytical balance (Sartorius, Germany); AS3100 interactive LCD digital microscope (Shenzhen Aike Science Education Technology Co., Ltd.); XB.K.25 hemocytometer (Shanghai Qiujing Biochemical Reagent & Instrument Co., Ltd.); 10127105P-G (25 mm × 75 mm) pathology-grade microscope slides (Jiangsu Shitai Laboratory Equipment Co., Ltd.); 10212424C (24 mm × 24 mm) microscope cover slips (Jiangsu Shitai Laboratory Equipment Co., Ltd.); 25.4 mm × 76.2 mm concave slides (Jiangsu Feizhou Glass & Plastic Co., Ltd.); UV-3900 UV-visible spectrophotometer (Hitachi, Japan); CR22N floor-standing high-speed refrigerated centrifuge (Hitachi, Japan); PYX-250S-C biochemical incubator (Shaoguan Keli Experimental Instrument Co., Ltd.); DDHZ-300 constant temperature shaking incubator (Taicang Experimental Equipment Factory); BSD-YX3200 constant temperature vertical double-layer intelligent shaker (Shanghai Boxun Medical Biological Instrument Co., Ltd.); HVE-50 high-pressure sterilizer (Hirayama, Japan); JIDI-5D multipurpose benchtop low-speed centrifuge (Guangzhou Jidi Instrument Co., Ltd.) [1].

2.3 Experimental Methods

2.3.1 In Vitro Antibacterial Characteristics

Mycelial Growth Inhibition Assay: The inhibition effect of tea essential oil microemulsion on Colletotrichum musae was determined based on the mycelial growth rate method (Aguilar-González et al., 2015). Microemulsion preparation: 9.0 g of Tween 80, 3.0 g of absolute ethanol, and 1.0 g of tea essential oil were mixed thoroughly with a glass rod, followed by the addition of 52.0 g of sterile water. Surfactant mixture preparation: A control treatment group was prepared using 9.0 g of Tween 80, 3.0 g of absolute ethanol, and 52.0 g of sterile water. Prochloraz preparation: A 25% prochloraz aqueous solution was prepared as a 250 mg/L stock solution and diluted to a 0.25 mg/L working solution. The prepared microemulsion, surfactant mixture, and prochloraz solution were separately mixed with PDA medium cooled to approximately 55°C. The final concentrations were as follows: Microemulsion and surfactant mixture: 5%, 10%, 15%, 20%, 25%, and 30%. Prochloraz: 0.025, 0.020, 0.015, 0.010, and 0.005 mg/L. A pure PDA plate served as the control. The PDA media with treatments and controls were poured into 9 cm sterile Petri dishes, with 10 mL per plate. Using a sterile pipette tip, 1 cm diameter fungal plugs were taken from the periphery of Colletotrichum musae colonies grown for 5 days and placed at the center of each plate. The plates were incubated in the dark at 28°C for 5 days. Colony diameters were measured, and each treatment was repeated three times[2].

2.3.2 In Vivo Antibacterial Characteristics

Spore Suspension Preparation: Following the method of Yang and Jiang (2015), 10 fungal plugs (1 cm diameter) were taken from the periphery of 7-day-old Colletotrichum musae colonies grown at 28°C and placed in 100 mL of PDB liquid medium. The culture was incubated at 28°C with shaking at 120 rpm for 3 days. A 25 mL aliquot of the culture was filtered through double-layer gauze into a 50 mL centrifuge tube and centrifuged at 4000 rpm for 10 minutes at room temperature. The supernatant was discarded, and the pellet was resuspended in 5 mL of fresh sterile PDB medium. Spore concentration was adjusted to 10^7 spores/mL using a hemocytometer and PDB medium. In Vivo Inoculation Assay: Following the method of Pang Xuequn (2008), individual banana fingers were washed with clean water and air-dried. The protective and therapeutic effects of tea essential oil microemulsion were tested as follows: Protective effect: Three circular holes (3 mm diameter) of uniform depth and spacing were drilled on the same surface of each banana. Excess moisture was removed. The bananas were immersed in 30%, 50%, and 100% microemulsion solutions, 250 mg/L prochloraz solution, or sterile water for 2 minutes, then air-dried. After 12 hours, 20 μL of the spore suspension was added to each hole. The bananas were placed in 0.04 mm polyethylene sealed bags (unsealed) and stored at 25 ± 2°C with 70%–80% relative humidity. After 10 days, lesion diameters were measured using a cross-intersection method. Each treatment included 6 bananas, with 4 replicates. Therapeutic effect: The same procedure was followed, except that after drilling the holes, 20 μL of spore suspension was added and absorbed for approximately 12 hours before immersing the bananas in 4596.5 mg/L, 7661 mg/L, and 15322 mg/L microemulsion solutions, 250 mg/L prochloraz solution, or sterile water for 2 minutes. Storage conditions

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were the same as in the protective effect test [2].

3. Results and Analysis

3.1 In Vitro Antifungal Activity

To evaluate the inhibitory effect of tea tree essential oil microemulsion on *Colletotrichum musae*, this study used the commonly applied banana preservative prochloraz as a positive control. The inhibitory effect of different concentrations of microemulsion on *C. musae* mycelial growth was determined. The results showed that the positive control, prochloraz, exhibited varying levels of inhibition on *C. musae* within the concentration range of 0.025, 0.020, 0.015, 0.010, and 0.005 mg/L (Figure 1). Furthermore, the inhibition rate increased with higher concentrations, following a logarithmic regression equation between prochloraz concentration and the probability value of mycelial inhibition rate: $y = 3.4222x + 11.643$ ($R^2 = 0.9899$). A concentration-dependent effect was observed. After five days of treatment, the 30% microemulsion exhibited a mycelial growth inhibition rate of 77.9%, which was close to the inhibition rate of 78.4% observed for 0.020 mg/L prochloraz. Meanwhile, within the tested concentration range, the surfactant alone showed no significant inhibitory effect on *C. musae* (Figure 1) [3].

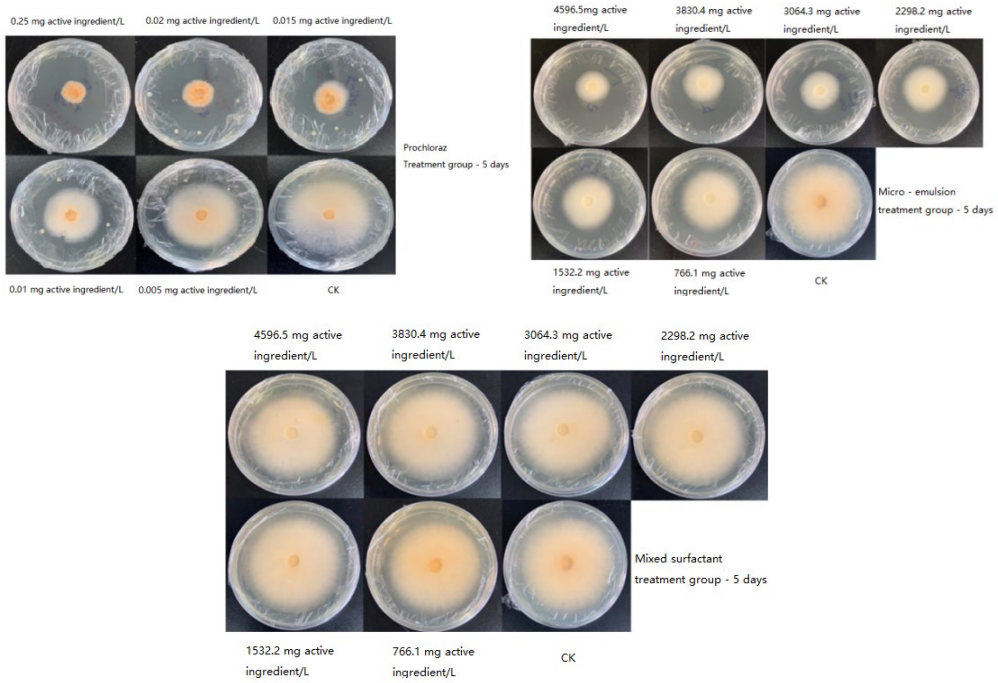

Figure 1: The inhibition effect of microemulsions, mixed surfactant and prochloraz to mycelial growth of Colletotrichum musae

3.2 In Vivo Antifungal Activity

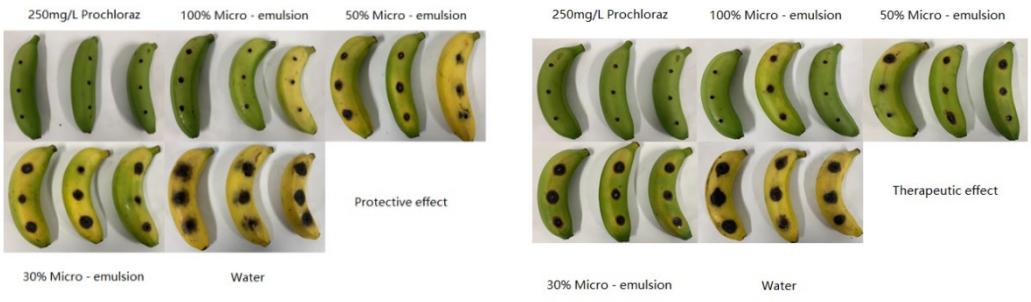

Figure 2: The control effect of microemulsions and prochloraz to Colletotrichum musae

As shown in Figure 2 and Table 1, after 10 days of artificial inoculation with *Colletotrichum musae*,

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the distilled water control group exhibited significant disease symptoms, with lesion diameters ranging from 18.50 to 19.78 mm. Following treatment with 30%, 50%, and 100% microemulsions, as well as 250 mg/L prochloraz, the severity of banana anthracnose was significantly lower than in the distilled water control group, regardless of whether the treatment was applied as a protective or therapeutic measure. The lesion diameters were significantly smaller than those in the control group, indicating that both the tea tree essential oil microemulsion and the positive control (prochloraz) had noticeable protective and therapeutic effects against banana anthracnose.Among the three tested microemulsion concentrations, the disease control efficacy increased with increasing microemulsion concentration. The 30% microemulsion exhibited a control efficacy of 26.1% to 39.4%, while the 50% microemulsion achieved an efficacy of 59.6% to 60.1%. The 100% microemulsion demonstrated the highest efficacy, comparable to that of 250 mg/L prochloraz, achieving a disease control rate of over 88% [4,5].

Table 1: The control effect of microemulsions and prochloraz to Colletotrichum musae

Antibacterial effect	Treatment	Pathogen diameter(mm)	Average control effect(%)
Protective effect	0(CK)	18.50±4.98^{a}	0^{e}
	30% Micro - emulsion	11.22±3.91^{c}	39.4^{c}
	50% Micro - emulsion	7.39±3.34^{d}	60.1^{b}
	100% Micro - emulsion	0.44±0.50^{e}	97.6^{a}
	250mg/L Prochloraz	0.33±0.47^{e}	98.2^{a}
Therapeutic effect	0(CK)	19.78±3.05^{a}	0^{e}
	30% Micro - emulsion	14.61±2.78^{b}	26.1^{d}
	50% Micro - emulsion	8.00±4.56^{d}	59.6^{b}
	100% Micro - emulsion	2.22±3.15^{e}	88.8^{a}
	250mg/L Prochloraz	1.33±1.05^{e}	93.3^{a}

Note: Different lowercase letters indicate significant differences in values within the same column ($P<0.05$).

4. Conclusion

This study evaluated the in vitro and in vivo antifungal activity of tea tree essential oil microemulsion against *Colletotrichum musae*. The results demonstrated the following:

(1) After 5 days of treatment, the 30% microemulsion achieved a mycelial growth inhibition rate of 77.9%, which was comparable to the inhibition rate of 0.020 mg/L prochloraz (78.4%).

(2) The in vivo antifungal activity experiments indicated that the tea tree essential oil microemulsion exhibited both protective and therapeutic effects similar to prochloraz. The 100% microemulsion demonstrated the highest efficacy against *C. musae*, achieving a disease control rate of over 88%, which was comparable to the efficacy of 250 mg/L prochloraz.

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