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CONTROL OF *ALTERNARIA* ROT OF CHERRY TOMATOES BY FOOD-GRADE *LAURUS NOBILIS* ESSENTIAL OIL MICROEMULSION

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ABSTRACT

Essential oils were widely reported to control the postharvest diseases of fruits. However, the stability and solubility limited the application of essential oils. The microemulsion loaded with *Laurus nobilis* oil was prepared with the optimal formula: oil/Tween 20/ethanol: 3/8/9, which was food-grade with the ability to be unlimitedly diluted with water and stable under high speed centrifugation and longtime storage. The *in vitro* test indicated that microemulsion showed great antifungal activity with the minimal inhibitory concentration and minimal fungicide concentration 500 μg mL^{-1} and 1000 μg mL^{-1}. The *in vivo* test showed that 500 μg mL^{-1} microemulsion efficiently protected cherry tomatoes (*Lycopersicon esculentum*) from decaying. Microemulsion controlled the postharvest disease via damaging the cell membrane of pathogen and increasing resistance related enzymes activity. This work suggested that the oil/Tween 20/ethanol microemulsion could be used as a carrier to load *L. nobilis* oil, which had efficient antifungal activity *in vitro* and *in vivo*.

PRACTICAL APPLICATIONS

The formula of the microemulsion developed in this study solved the problem of solubility and stability of *Laurus nobilis* essential oil, which enhanced the antifungal activity of essential oil. The microemulsion could be used as a fungicide to control the *Alternaria* rot of cherry tomatoes.

INTRODUCTION

Cherry tomato (*Lycopersicon esculentum*) is a kind of common fruit or vegetable in China as well as in many other countries. Cherry tomato is sensitive to many pathogenic fungi such as *Alternaria* and *Aspergillus* for the reason of the soft pericarp (Tian et al. 2011). The pathogenic fungi grow quickly during storage time at appropriate temperature and relatively high humidity. Chemical fungicides, for example, carbendazim, tebuconazole and azoxystrobin (Steinbach and Stevens, 2003) are commonly used to inhibit the growth of pathogens and control the postharvest diseases. However, the toxicity to human beings or animals, the resistance to pathogens and the pollution to the environment limit the application of chemical fungicides (Soylu et al. 2006).

Essential oils, which are extracted from plant flowers or leaves, are becoming a new way to control postharvest diseases. It was reported that essential oils, such as thyme (*Thymus vulgaris*) oil, cassia (*Cinnamomum cassia*) oil, origanum (*Origanum vulgare*) oil, and so forth. showed significant effect on controlling postharvest diseases of fruits and vegetables (Soylu et al. 2006; Feng and Zheng, 2007; Antunes and Cavaco, 2010; Vitoratos et al. 2013).

Bay (*Laurus nobilis*) essential oil, extracted from the leaves of bay tree, also has great antibacterial and antifungal activities at low concentration, which can be used as a potential biocontrol agent (Simić et al. 2004; De Corato et al. 2010). The components of the essential oil were investigated in the previous work, and it was proved that eugenol, caryophyllene and cinnamaldehyde were the main components of the

essential oil which played important roles in antifungal activity (Xu et al. 2014). However, the poor solubility in water, the instability and volatility during storage and usage limit the application of essential oils.

Microemulsions, different from normal emulsions, are thermodynamically stable, transparent, low viscosity and isotropic dispersions colloidal nanodispersions, which have potential usage as delivery systems for enhancing the solubility and protecting the biological activity of some substances (Al-Adham *et al.* 2000; Alam *et al.* 2010; Zhang *et al.* 2010). To our knowledge, few articles concentrated on the application of microemulsion to control the postharvest diseases of fruit and vegetable. It was reported that microemulsion system loading antimicrobial agents had significant effect on bacterial and fungal cells over short time scales. Zhang *et al.* (2008, 2009) developed a food-grade microemulsion loading with the antimicrobial agent: glycerol monolaurate, which showed the antimicrobial activity against bacteria, yeasts and molds. In our previous work, we succeeded in developing a microemulsion using Tween 20, ethanol and water, loading with thyme oil, which showed excellent antifungal activity against *Geotrichum citri-aurantii* both *in vitro* and *in vivo* (Xu et al. 2012).

The object of this article is to develop a new microemulsion loading with *L. nobilis* oil, which has already been proved to have antifungal activity, detect the dilutability and stability of the prepared microemulsion, evaluate the antifungal activity by the methods of agar diffusion, minimal inhibitory concentration (MIC) and minimal fungicide concentration (MFC) determination and conidia germination *in vitro*, scan with electron microscope to see the interaction mechanism and apply on wounded cherry tomatoes *in vivo*. It is expected that the *L. nobilis* oil microemulsion can efficiently inhibit the growth of *A. alternata in vitro* and *in vivo*.

MATERIALS AND METHODS

Chemicals

Pure grade essential oil (without adding chemical synthesis agents) of *L. nobilis* origin in China was purchased fromYu Yue Essential Oil Market (Guangzhou, Guangdong, China). The essential oil was stored in dark bottles at 4C. Tween 20 (AR), Tween 80 (AR), ethanol (AR), dextrose (AR) and agar were purchased from Sinopharm Chemical Reagen Co., Ltd (Shanghai, China).

Fungus and Cultures

Alternaria alternata was isolated from the local decayed cherry tomatoes and cultured on potato dextrose agar medium (PDA: extract of 200 g boiled potatoes, dextrose, 20 g; agar, 20 g; and deionized water, 1,000 mL) at 28C for 7 days.

Phase Diagrams

The behavior of microemulsions was described on a pseudo-ternary phase diagram. The phase diagrams were drew with the method of Zhang *et al.* (2009) with modification. Tween 20 (surfactant) and ethanol (cosurfactant) were mixed in glass test tubes sealed with caps. *L. nobilis* oil (oil phase) was then added into the mixture at the predetermined weight ratios of oil phase to surfactant and kept at 25C. The distilled water (aqueous phase) was titrated into the oil-surfactant-cosurfactant mixture to determine the microemulsion area. All samples were vigorously stirred and treated with ultrasound for 30 min. After equilibrating at 25C for at least 24 h, the samples were examined. The different phases were differed using ocular and optical (crossed polarizers) methods. The sample remained transparent and homogeneous after vigorous vortexing and ultrasound was regarded as a monophasic area. The accuracy in the location of the phase boundaries was within 4 wt %. The total monophasic region, denoted A_D, was used to describe the solubilization capacities of *L. nobilis* oil microemulsions (Garti *et al.* 2001; Yaghmur *et al.* 2002).

Dilutability and Stability

The samples were diluted with 10 to 1,000 times distilled water to determine the dilutability, centrifuged at 3,000–5,000 rpm and stored for 30 days to determine the stability.

In vitro Antifungal Assay

Agar Diffusion Test. Microemulsion was added into the PDA medium which was autoclaved and cooled in a water bath to 50C to obtain the final concentrations (200, 400, 600, 800, 1,000 μg mL^{-1}), and sterile water without microemulsion was used as control. The mixed PDA medium was poured into 90 mm petri plates with 20 mL for each plate. A 6 mm plug of pathogens was cut from a plate of pathogen which was cultured for 7 days. The plugs were inoculated in the center of plates. At least five replicates were performed per treatment. All the pathogens were incubated at 28C. The diameters of plaques were measured by the crossing method every day. The inhibition rate was calculated as the percentage of inhibition of radial growth relative to the control. Experiments were performed three times.

MIC and MFC Determination. Minimum inhibitory concentration and MFC were determined by broth dilution method in test tubes (Batta, 2004). Microemulsion was added into a 15 mL glass tube containing 5 mL potato dextrose broth (PDB) medium to obtain the final concentrations (125, 250, 500, 1,000, 2,000 μg mL^{-1}), and sterile

water was used as control. Hundred microliter spore suspensions (1×10^7 spores mL^{-1}) of the pathogen were added into each tube. After being incubated at 28C, 200 rpm, for 48 h, the tubes were observed. The lowest concentration showing no visible growth was regarded as the MIC. Spores from the tubes which showed no visible growth were transmitted to PDA medium to determine the inhibition was temporary or permanent. The lowest concentration at which no growth occurred on PDA medium was defined as MFC.

Conidial Germination. Microemulsion was added into a 15 mL glass tube containing 5 mL PDB medium to obtain 1,000 μg mL^{-1}, and sterile water was added as control. Hundred microliter spore suspensions (1×10^7 spores mL^{-1}) of the pathogen were added into each tube. Conidia were incubated at 28C, on a rotary shaker (200 rpm). After 20 h incubation, at least 200 conidia per replicate were observed microscopically to check whether germinated. The germination ratio was calculated by the formula:

$$\text{Germination ratio} = \frac{\text{Germinated conidia number}}{\text{Total observed conidia number}} \times 100\%$$

Three replicates were used per treatment, and experiments were performed three times (Feng and Zheng, 2007).

Hyphal Morphology Observation by SEM

Fifty milliter of autoclaved PDB medium was placed in sterile Erlenmeyer flask, added with microemulsion to obtain 500 μg/mL, and microemulsion without *L. nobilis* oil was used as control. Hundred microliter spore suspensions (1×10^5 spores/mL) of pathogens were added into each flask. The flasks were then incubated for 14 days at 28C on a rotary shaker (200 rpm). After 10 min refrigerated centrifuge, the precipitates were obtained for the SEM analysis.

For SEM analysis, the fungal mycelia were treated as follows: precipitated mycelia (1 g) were mixed with 2.5% glutaraldehyde which was diluted with 0.1 M phosphate-buffer (pH = 7.0) over night at 4C. Then the mycelia were washed by 0.1 M phosphate-buffer (pH = 7.0) for 3 times, each time for 30 min. After fixation by 1% osmic acid for 2 h, they were dehydrated in a graded ethanol series (50, 70, 80, 90, 95, 100 and 100%) for 15 min separately. Then the samples were washed by isoamyl acetate for 2 h. The processed samples were observed by SEM (S-3000N, Hitachi Limited, Japan).

In vivo Antifungal Assay

The commercially grown cherry tomatoes from Fujian, China, in the same size, same maturity, without any injuries and infections, were selected for the experiments. The entire test fruits were dipped in the 0.1% sodium hypochlorite solution for 2 min, and then rinsed with tap water, air dried in 1.5 L plastic boxes (20 cherry tomatoes per box).

Fruits were wounded with a sterile puncher to form a uniform wound (6 mm diameter, 2 mm deep) at the equatorial region. Twenty microliter of different concentrations (500 and 1000 μg ml^{-1}) of *L. nobilis* oil microemulsion was pipetted into each wound, and the sterile water was pipetted as control. At the same time, 30 μL of conidial suspension of the pathogen (5×10^4 spores mL^{-1}) were pipetted into each wound. The treated tomatoes were stored at 20C, and the rate of decayed fruits was calculated every day. There were three replicates for each treatment with 20 fruits, and the experiments were repeated twice.

Resistance Related Enzymes Activity

Cherry tomatoes were wounded and treated with 30 μL 500 μg mL^{-1} essential oil or microemulsion. Sterile water was used as control. Samples were stored at 25C and taken at different time points (0, 12, 24, 48 and 72 h) after treatment. After peeling, the fresh tissue (about 0.6 g) of each treatment around the wound was ground in a mortar and pestle with 5 mL of cold (4C) 50 mmol L^{-1} sodium phosphate buffer (PBS; pH 7.8) containing 1.33 mmol L^{-1} EDTA and 1% (w/v) polyvinyl polypyrrolidone. The homogenates were centrifuged at 4C and 12,000 rpm for 15 min, and the supernatants were collected for assay.

Protein content was determined following the method described by Bradford, (1976) with bovine serum albumin as the standard. Three replicates were performed and 20 tomatoes were used in one replicate.

POD activity was measured using guaiacol as a substrate following the method described by Lurie and Wolfe (1997) with modification. 3 mL of 50 mmol L^{-1} PBS (pH 6.4), 220 μL of 0.3% (v/v) guaiacol, 60 μL of 0.3% (v/v) H_2O_2, and 20 μL of crude enzyme extract were mixed. The reaction was initiated immediately by adding H_2O_2 at 30C. After 5 min reaction, absorbance at 470 nm was measured every 30 s. One unit of the POD activity is defined as the amount of the enzyme extract producing an increase of A470 nm by 0.01 in 1 min and expressed as U per mg protein.

PAL activity was measured following the method of Camm and Towers (1973) with modification. 1 mL crude enzyme extraction was mixed with 4 mL Borate saline buffer (pH 8.8), containing 10 mmol L^{-1} phenylalanine. After incubated at 30C water bath for 30 min, absorbance at 290 nm was measured every 30 s. One unit of the PAL activity is defined as the amount of the enzyme extract producing an increase of A290 nm by 0.01 in 1 h and expressed as U per mg protein.

PPO activity was measured following the method of Aquino-Bolaños and Mercado-Silva (2004) with modification. The reaction mixture contained 290 μL of 50 mmol L^{-1}

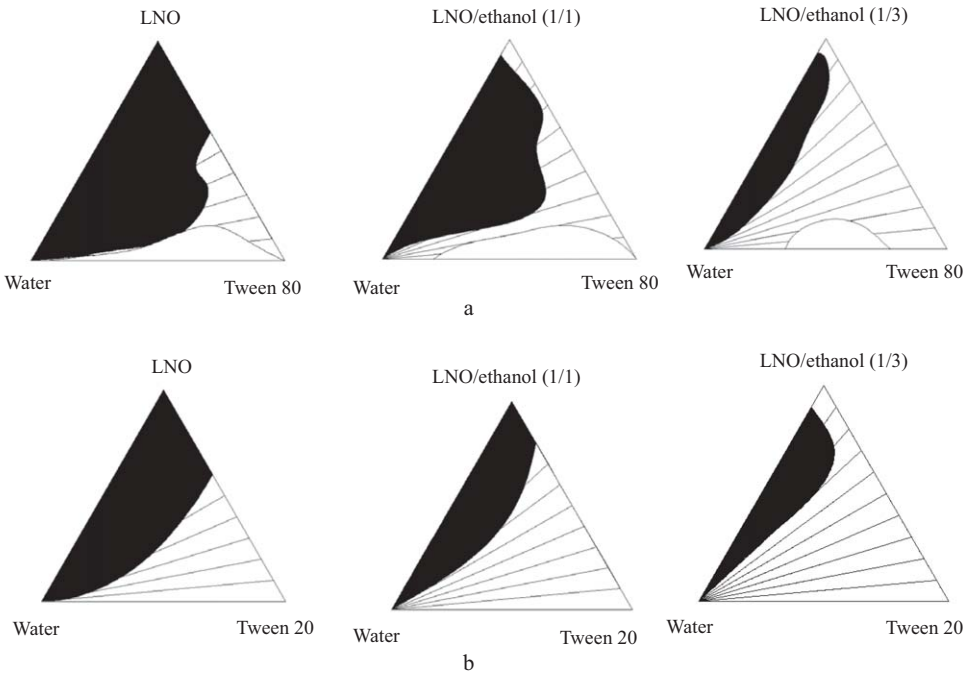

FIG. 1. THE PSEUDOTERNARY PHASE DIAGRAM OF DIFFERENT FORMULAS FOR FOOD-GRADE *L. NOBILIS* OIL MICROEMULSION

PBS (pH 6.4) containing 100 μmol L^{-1} catechol as the substrate, and 10 μL of crude enzyme extract. After 5 min reaction, the absorbance at 398 nm was measured every 30 s. One unit of the PPO activity is defined as the increase of 0.01 in A398 per min, and expressed as U mg^{-1} protein.

Statistical Analysis

All the data reported as mean standard deviations. Significant differences between the mean values were determined by Duncan's Multiple Range test ($P < 0.05$), following one-way ANOVA. The statistical analysis was performed with the software of SPSS, 13.0 (Chicago, USA).

RESULT

Formulation of Microemulsion

The phase diagrams of the microemulsion consisted of *L. nobilis* oil/Tween 80/ethanol/water were listed in Fig. 1a, which was obviously that without ethanol, the single phase was small ($A_T = 15.4\%$) (Fig. 2), large area of LC (liquid crystal) phase appeared, and no diluting channels exist. When the ratio of oil to ethanol was 1:1, the microemulsion phase area increased and the diluting channels appeared. When oil/ethanol = 1:3, the single phase area continued increasing and the LC phase diminished drastically, and the microemulsion can unlimited dilute at oil/Tween 80 = 6:4. The single phase area of the formula of oil/Tween 20/water (44.6%) was significantly larger than oil/Tween 80/water (15.4%) (Figs. 1a,b and 2). It was similar to the Tween 80 group that the addition of ethanol significantly increased the microemulsion phase area and broadened the diluting channels. And the optimal formula was oil/Tween 20/ethanol: 3:8:9.

Dilutability and Stability

The formulation of *L. nobilis* oil/Tween 20/ethanol = 3:8:9 could be diluted with distilled water unlimitedly. Furthermore, this formulation kept single phase under high speed centrifugation (5,000 rpm) and longtime storage (30 days),

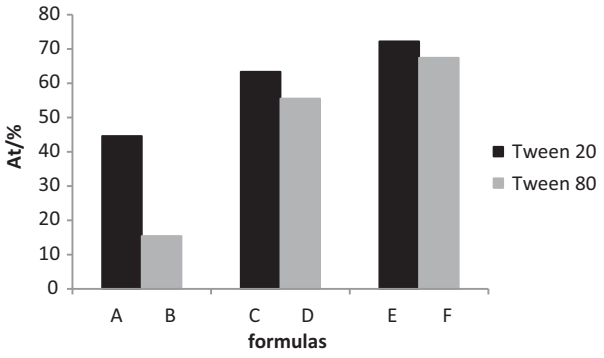

FIG. 2. THE PERCENTAGE OF AREAS OF SINGLE-PHASE REGION OF DIFFERENT FORMULAS

TABLE 1. THE DILUTABILITY AND STABILITY OF MICROEMULSION

	Dilution ratio			Storage time/day			Rotate speed/rpm		
	10×	100×	1000×	1	10	30	3,000	4,000	5,000
Microemulsion	T	T	T	T	T	T	T	T	T

Note: Letter "T" means transparent.

which built the foundation for the further application of the microemulsion (Table 1).

Agar Diffusion Test

The antifungal activity of the microemulsion formed with *L. nobbilis* oil/Tween 20/ethanol = 3:8:9 was determined by the methods of agar diffusion (Fig. 3). The result showed that 200 μg mL^{-1} microemulsion significantly inhibited the growth of *A. alternata* after 48 h, while 800 μg mL^{-1} microemulsion extended the initial growth time. When treated with 1,000 μg mL^{-1} microemulsion, no visible pathogens grew on the agar medium.

MIC and MFC Determination

The MIC and MFC of the microemulsion against *A. alternata* were determined. It was figured out that the growth could not be obviously observed at the concentration of 500 μg mL^{-1}, which was considered as the MIC (Table 2). However, when transferred to a new PDA plate without microemulsion, the pathogen grew again, which mean the low concentration (500 μg mL^{-1}) of microemulsion could only inhibit the growth of pathogen, not directly kill it. As to high concentration (more than 1,000 μg mL^{-1}), the pathogen showed no growth on the new PDA plate, which implied that high concentration of microemulsion showed the fungicidal activity.

Conidia Germination

Conidial germination of the tested pathogen was strongly inhibited in the presence of microemulsion (Fig. 4). At 250 μg mL^{-1} the germination rate was 71.6%, while at 1,000 μg mL^{-1}, the rate dramatically decreased to 11.3%, which indicated that the concentration of the microemulsion significantly influenced the germination-inhibiting ability.

Hyphal Morphology Observation

It was obvious that after treated with the microemulsion at the concentration of 500 μg mL^{-1}, no visible spores in visual field (Fig. 5a,b). The mycelia showed dramatic morphology alteration compare to the control, with the fold and drape appeared (Fig. 5c,d).

In vivo Antifungal Assay

It was indicated in Fig. 6 that the decayed rate of tomatoes reduced significantly after treated with microemulsion for

TABLE 2. THE MINIMAL INHIBITORY CONCENTRATION (MIC) AND MFC OF *L. NOBILIS* OIL MICROEMULSION

Concentration (μg ml^{-1})	125	250	500	1,000	2,000
MIC	++	++	−	−	−
MFC	++	++	++	−	−

Note: Symbol "++" means large amount growth of *A. alternata*, symbol "+" means visible growth of *A. alternata*, symbol "−" means no visible growth of *A. alternata*.

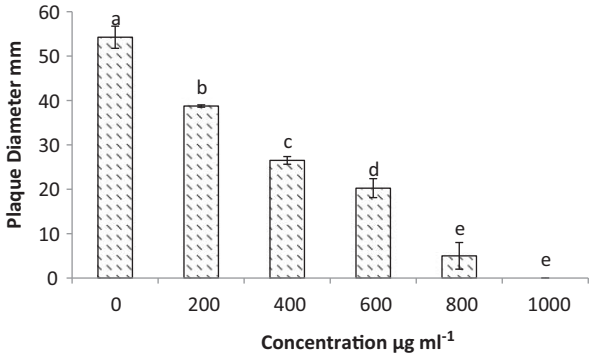

FIG. 3. EFFECT OF DIFFERENT CONCENTRATION (0, 200, 400, 600, 800, 1,000 MG ML^{-1}) OF *L. NOBILIS* MICROEMULSION *IN VITRO* AGAINST *ALTERNARIA ALTERNATA*

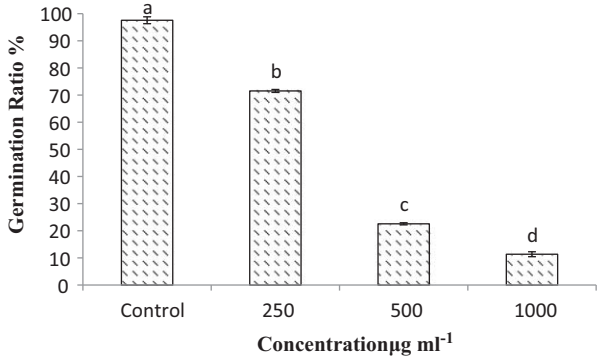

FIG. 4. EFFECT OF DIFFERENT CONCENTRATIONS (250, 500, 1,000 MG ML^{-1}) OF *L. NOBILIS* OIL MICROEMULSION ON THE GERMINATION OF *A. ALTERNATE* SPORES

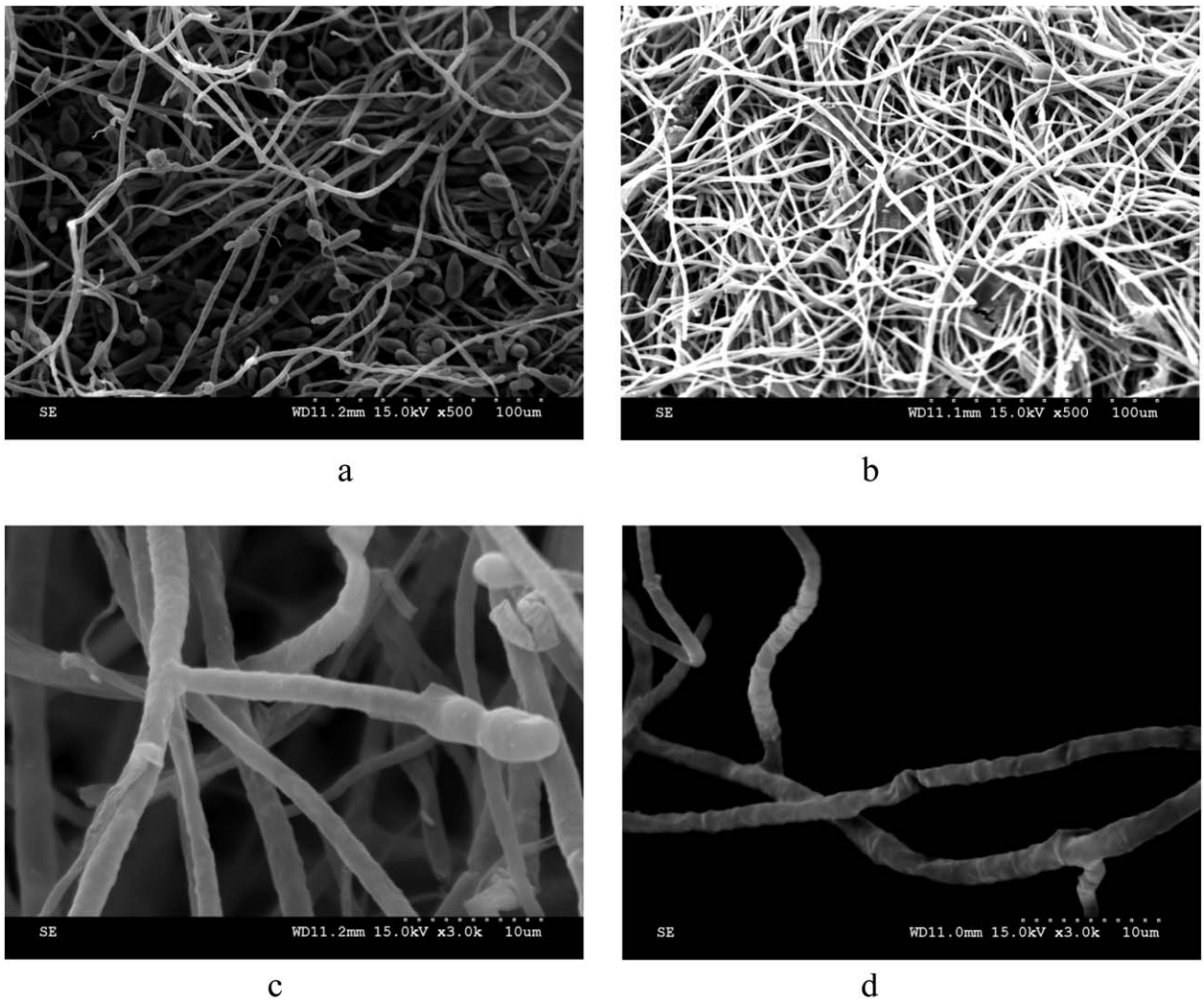

FIG. 5. THE EFFECT OF DIFFERENT CONCENTRATIONS (250, 500, 1,000 MG ML^{-1}) OF *L. NOBILIS* OIL MICROEMULSION ON THE GERMINATION OF A. *ALTERNATA*

96 h at 25C. At the concentration of 500 μg mL^{-1}, the decay rate was inhibited to 46.7%, as to 1,000 μg mL^{-1}, only 13.3% tomatoes decayed. As the main composition of the microemulsion, ethanol and Tween 20 was known as a kind of bactericide. However, the ethanol and Tween 20 showed no significant antifungal activity at the concentration of 1,000 μg mL^{-1} *in vivo*.

Resistance Related Enzymes Analysis

POD Activity. According to the result in Fig. 7a, in the first 36 h, there was no obvious difference between microemulsion, essential oil and control. When it came to the 48th hour, the POD activity of tomatoes treated with microemulsion increased dramatically. While the POD activity in essential oil group was still low and started to increase until to

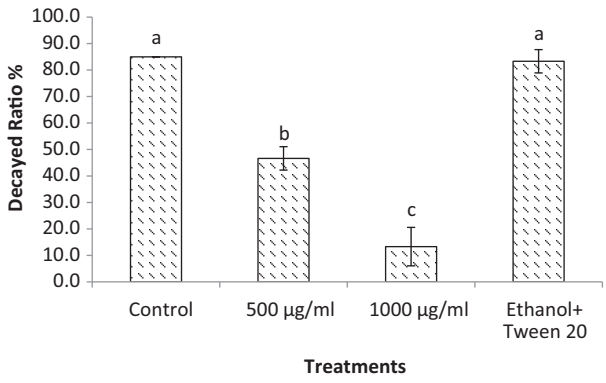

FIG. 6. SCANNING ELECTRON MICROSCOPY OF HYPHAE EXPOSED TO *L. NOBILIS* OIL MICROEMULSION

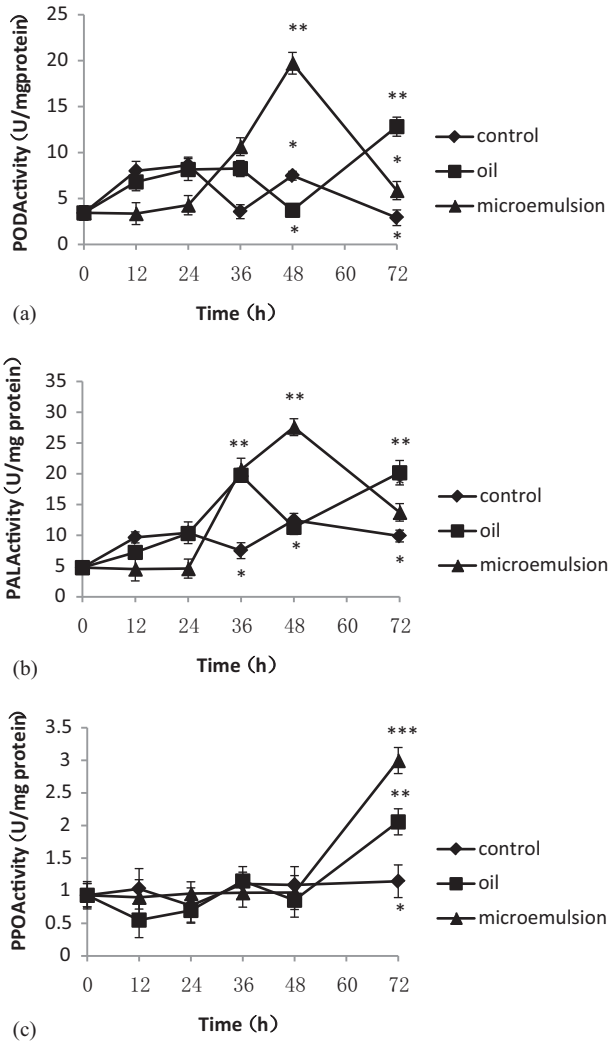

FIG. 7. THE EFFECT OF *L. NOBILIS* OIL MICROEMULSION AGAINST *A. ALTERNATA* IN CHERRY TOMATOES (96 H)

72nd hour. The POD activity in control group kept in a relative low level in this period.

PAL Activity. Similar to the POD activity result, at the beginning of treatment, there was no difference between groups. At the 36th hour, PAL activity in microemulsion group and essential oil group increased, significantly higher than control group ($P < 0.05$). Then the microemulsion group continuously went up and the essential oil group came down in the 48th hour.

PPO Activity. At the first 48 h after treatment, the PPO activity in three groups all kept stable. In the 72nd hour, PPO activity in the microemulsion group increased the most quickly, and the essential oil group also increased significantly compare to the control ($P < 0.05$).

DISCUSSION

In recent years, one of the nanotechnologies, microemulsion, is gradually applied in food preservation. It was widely reported that microemulsion loaded with essential oil or bacteriostatic agent showed significant antibacterial activity (Batta, 2004; Kitamoto *et al.* 2009). Triton X-100, Tergitol, n-hexanol and isopropyl myristate were used as surfactant and cosurfacant in many reported microemulsion formulas, which were not food-grade (Al-Adham et al. 2013; Badawi *et al.* 2009; Deshpande *et al.* 2008). As a result, these microemulsions could not be applied in food industry as a kind of preservative. In this study, a new microemulsion was developed with food-grade constitutes. It was proved that this microemulsion showed great stability under long-time storage, vigorously vortex and dilution, which laid the foundation for further application of the microemulsion.

The results demonstrated that the microemulsion using Tween 20 as surfactant showed more single phase area and no liquid crystal area in the pseudo-ternary phase diagram which proved to have better emulsifiability than tween 80. The similar results were also showed in some other studies, with the explanation that Tween 20 (HLB = 16.7) is more hydrophilic than Tween 80 (HLB = 15), which helps to increase the interfacial fluidity and flexibility of the surfactant film (Von Corswant and Söderman, 1998). The addition of ethanol significantly extended the area of single phase and enabled the appearance of the infinite dilution line. The main explanation was that the addition of cosurfactant changed the film properties. The flexibility of the film formed by the oil phase, aqueous phase and surfactant is increased, to enhance the interfacial fluidity for the formulation of isotropic microemulsion phase when added with short-chain alcohols, as a result, the liquid crystal phase is destabilized to form microemulsion phase (Zhang et al. 2007), and the cosurfactant is incorporated into the surfactant film, which lowers the bending moduli to form more microemulsion area (Garti et al. 2001; Gradzielski, 1998).

In vitro results indicated that the microemulsion treatment could inhibit the growth and conidia germination of *A. alternata* at low concentration ($<800\ \mu g\ mL^{-1}$) while at relatively high concentration ($>1000\ \mu g\ mL^{-1}$) the pathogens were killed at a short time, This conclusion was similar to the reports of other essential oil microemulsions (Nirmala et al. 2013; Wang et al. 2014). Commonly, the antifungal activity of essential oil loaded in the microemulsion was better than essential oil separately. There are three possible explanations flowing: (1) Increasing the solubility of *L. nobilis* oil. The essential oil had poor solubility in water, which limited the contact between essential oil and pathogen cell membrane. In common sense, essential oil kills pathogens by interacting with their cell membranes. The microemulsion provided a thermal stability water-dilutable system for the

interaction between essential oil and pathogen cells; (2) Improving the stability of *L. nobilis* oil. It was reported that essential oil had great antifungal activity at the beginning, while the effect decreased fast in the following weeks due to the volatilization of essential oil (Lawrence and Palombo, 2009). Microemulsion could efficiently prevent essential oil volatilizing (result was not shown), which leaded to a longer action time; and (3) Damaging the cell membrane. Al-Adham *et al.* (2013) reported that microemulsion had the ability to destroy the plasma membrane for the reason that microemulsion had stronger emulsifying capacity than normal emulsion, which meant it could easier dissolve the phospholipids membrane.

The result of conidia germination showed that conidia germination was dramatically inhibited at 1,000 μg mL^{-1}, but some conidia still germinated at the MFC, which seemed to be antagonistic to the result of MFC. The possible explanation was that although the germination was not completely inhibited, the growth of mycelia was controlled when the presence of the microemulsion. As a result, there were no visible mycelia or colony during incubation. This was similar to the publication of Tian *et al.* (2012), who found that essential oil efficiently decreased the mycelia weight of pathogens.

Two possible explanation of the interaction mechanism between the pathogens and the microemulsion was found from the result of SEM. First, the microemulsion destroyed the structure of fungi directly, which influenced the metabolism, nutrition and energy exchange of the fungi, lead to the leak of the cytoplasm and made the cell die. Second, it inhibited the formation of the spores, which cutoff the reproductive line of the pathogen.

Essential oils were widely reported applied in the fruits for the postharvest preservation. However, the *in vivo* fungicide concentrations were usually much higher than *in vitro* concentration, because of the complex chemical constituent and volatility of essential oil (Soylu et al. 2006). In this research, we found that the *L. nobilis* oil microemulsion had similar antifungal activity *in vivo* and *in vitro* at the same concentration, which may cause by two possible reasons. One is because the microemulsion prevented the essential oil from volatilization. It was known that smaller particle makes emulsion more stable. The microemulsion reduced the particle size to about 10 nm, which acted as a sustained-release system to make the essential oil stay longer on the surface of fruits. The other explanation is that the microemulsion activated the disease-defense system of the tomato, while the essential oil alone did not have this function. POD, PAL and PPO are enzymes which reported associated with fruit postharvest disease resistance (Deng *et al.* 2015; Tomas-Barberan and Espin, 2001; Zhang et al. 2015). According to the result, it was obvious that when treated with microemulsion, all these enzymes showed a significant increase.

CONCLUSION

The microemulsion with the formula, oil/Tween 20/ethanol: 3/8/9, loaded with *L. nobilis* oil, which enhanced the stability and solubility of *L. nobilis* oil, showed excellent antifungal activity against *A. alternata in vivo* and controlled the postharvest decayed in cherry tomatoes. The main interaction mechanism among pathogen, fruit and microemulsion was that the microemulsion destroyed the cell membrane of the pathogens and activated the disease-defense system of the fruits.

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