

## EVALUATION OF THE SYNERGISTIC EFFECT OF ETHANOL AND LEMONGRASS OIL AGAINST *Aspergillus niger*

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### ABSTRACT

The aim of this work was to evaluate the antifungal efficiency of ethanol and lemongrass oil at the different concentrations, alone and in combination both against *Aspergillus niger* by agar disk diffusion method, mycelial growth inhibition and broth dilution method to determine MIC and MFC. The ultraviolet (UV) absorption and electrical conductivity of the culture supernatant were used to determine membrane integrity. Scanning electron microscopy (SEM) performed to observe *A. niger* spore morphology. The results showed that the MIC and MFC values of lemongrass oil (in tween 20 0.3% v/v) was 5 µL/mL and 250 ppm which were 100 and 200 times respectively lower than ethanol. The combination of lemongrass oil in tween 20 with ethanol (20% v/v) show the best result with the inhibition zone and MGI (%) was observed at 2.5 µL/mL and 100% respectively. The UV absorption and electrical conductivity values increased quickly after 16h incubated in the antimicrobial agents whereas there were almost unchanged in the control at the point of time. The SEM results show that, the morphological changes of the *A. niger* spores due to significant wrinkles and distortion in the samples treated by lemongrass oil or lemongrass oil combining with ethanol while the *A. niger* spores in ethanol show slight wrinkles. These suggest that ethanol has a synergic effect which enhances the antifungal activity of lemongrass oil. This combination is necessary to ensure the antimicrobial effect as well as reduce the amount of used essential oil.

**Keywords:** Ethanol, lemongrass oil, antifungal activity, SEM, synergistic effect, tween 20

### INTRODUCTION

Post-harvest losses are one of the major causes of the loss of fresh vegetables during the supply chain (Nunes *et al.*, 2012). *Aspergillus niger* is one of the major causes of black rot of plain (Prakash *et al.*, 1988). Therefore, control of *A. niger* during the preservation is very necessary. The widespread use of synthetic fungicides for preserving agricultural products has significant limitations such as handling of hazards, pesticide residues, and risk to health and the environment (Dharini *et al.*, 2014). Current trends in antimicrobial agent research from the natural origin in which plant-derived essential oils are of great interest. In previous studies, lemongrass oil is considered an essential oil for safe and effective natural preservatives which has an effective antibacterial activity (Vazirian *et al.*, 2012). Essential oils are natural products consisting of a complex mixture of volatile molecules (Mahian *et al.*, 2016), which are liquid, soluble in organic solvents and insoluble in water (Bakkali *et al.*, 2008). The evaluation of the antimicrobial activity, the essential oils are often diluted at different concentrations by emulsifying agents (Burt, 2004). Hilbig *et al.* (2016) reported that emulsifier has the negative effect on the antimicrobial activity of essential oil (Hilbig, 2016), whereas the combination of ethanol and other antimicrobial agents (chitosan, potassium sorbate ...) show the antimicrobial activity better than using in single agent (Romanazzi *et al.*, 2007; Karabulut *et al.*, 2005). Besides essential oil, ethanol also showed antimicrobial effect (Gianfranco *et al.*, 2007). However, to achieve good antibacterial efficacy, ethanol is commonly used at high concentrations leading to increased production costs and the risk of fire safety (Ozgur *et al.*, 2005). Ethanol is made up of hydrophilic (-OH) and hydrophobic (CH<sub>3</sub>CH<sub>2</sub>-) radicals. Hydrophilic radicals (-OH) to help dissolve the polarizing elements and ions. Short chain CH<sub>3</sub>CH<sub>2</sub>-hydrocarbons can attract non-polar molecules. Therefore, the combination of ethanol and essential oil can both increase the dilution effect of the oil in the media and can synergize with the essential oil to enhance the effectiveness of the antimicrobial. Although the combination of ethanol and the essential oil is very promising, very few studies exist on the synergic effect of ethanol and lemongrass oil on fungi. In this study, the antifungal activity of lemongrass oil and ethanol used alone or in combination against *A. niger* was evaluated by agar disk diffusion method, mycelial growth inhibition and broth dilution method to determine MIC and MFC. The ultraviolet (UV) absorption and electrical

conductivity of the culture supernatant were used to determine membrane integrity. Scanning electron microscopy (SEM) performed to observe the morphology of *Aspergillus niger* spores.

### MATERIAL AND METHODS

#### Materials

*Aspergillus niger* M1 was obtained from strain collection of Faculty of Food Technology, Ho Chi Minh City University of Food Industry. *A. niger* was grown in PDA (Potato Dextrose Agar) medium at 30°C for 6 days. Then, the mass was harvested by rinsing plates with PDB (Potato Dextrose Broth). The freshly grown microbial cell at approximately 6 log CFU/mL was used for the evaluation of the antifungal activity.

The essential oil in this study was lemongrass oil (*Cymbopogon flexuosus*) from Tien Giang province, the City is located at 10°25'N 106°10'E in the southern region of Vietnam. Lemongrass was hand-collected and immediately used to obtain lemongrass oil by steam distillation. Lemongrass oil was stored in glass vials in the absence of light until gas chromatography analysis and to test its antifungal activity. The essential oil was directly analyzed by gas chromatography coupled to mass spectrometry (Agilent GC 7890B GC System, 7010 GC/MS Triple Quad). The used column was an HP-5MS (30 m long, 0.25 mm and 0.25 µm film thickness). The operating conditions were as follows: Helium was used as a carrier gas with a back pressure of 0.8 atm; flow rate of 1.0 mL/min; split 1:20; injection volume 0.2 µL; The injector temperature was 250°C and the oven temperature program started at 60 for 5 min and then increased at the rate of 5°C/min up to 150°C at 5°C/min, and increased from 150°C to 280°C at 10°C/min. The constituents in the essential oils were identified by computer matching of their mass spectral fragmentation patterns with those of compounds in the data bank NIST 98 and Wiley 275 library. Lemongrass oil and ethanol used alone or in combination with or without tween 20 (0.3% v/v) (an emulsifying agent) were used as antifungal agents for the next step.

**Evaluation of antifungal activity of lemongrass oil and ethanol used alone or in combination against *A. niger***

**Diffusion method**

The antifungal activity of lemongrass oil and ethanol was carried out according to Lieu *et al.* (2018a) with slight modifications. Briefly, the *A. niger* suspensions were spread over the surface of PDA (Potato Dextrose Agar) plates (at final concentration 6 log CFU/mL approximately) and allowed to dry in 5 min. Lemongrass oil (10; 20; 40; 80 and 100 µL/mL) and ethanol (10%; 20%; 40%; 80% and 100% v/v), used alone or in combination with or without tween 20 0.3% v/v (an emulsifying agent) were spotted on PDA agar (15 µL), that containing *A. niger* spores and tween 20 were used as controls. The plates were incubated at 30°C in 24 hours. After 24h incubated, Petri dishes were examined by inhibition zone.

**Determination of the antifungal effect of lemongrass oil and ethanol on mycelial growth**

The effect of emulsifying agents to antifungal activity of lemongrass oil on mycelial growth was carried out as assay previously described (Boubaker *et al.*, 2016) with slight modifications. Briefly, PDA supplemented individually with antifungal agents such as lemongrass oil; ethanol and the combination of lemongrass oil with ethanol. The mixture was poured into Petri plates. Afterward, the Petri plates were incubated with *A. niger* by spore culture at the middle of the Petri plates. The agar Petri plates were then incubated at 30°C in seven days. The control consisted of PDA medium supplemented with emulsifying agents without lemongrass oil. The antifungal activity was expressed in terms of percentage of mycelial growth inhibition (MGI) and calculated according to the following formula:

$$MGI (\%) = (C-T)/C \times 100\%$$

Where C and T represent mycelial growth diameter in control and lemongrass oil treated Petri plates, respectively

**Determination of MFC Using Broth Dilution Method**

Minimum fungicidal concentration (MFC) of lemongrass oil and ethanol were carried out according to the previous description (Lieu *et al.*, 2018a). A range of lemongrass oil (100÷1,000 µL/L in tween 20 0.3% v/v) and ethanol (10,000÷100,000 µL/L) concentrations use alone or in combination were prepared in PDB (Potato Dextrose broth) medium. Each flask was inoculated with 6 log CFU/mL of the *A. niger* spores. Flasks containing only tween 20 (without lemongrass oil) were used as the control. The flasks were incubated at 30°C in an orbital shaking incubator (100 rpm) for 48h. One mL of culture was taken from each flask (where growth was not observed) for serial dilution to make the inoculum of 6 log CFU/mL and inoculated on DRBC (Dichloran Rose Bengal Chloramphenicol) agar plates at 30°C in three days

**UV absorption and conductivity determination**

The experiments were conducted based on a previously published method (Suxia *et al.*, 2015) with slight modifications (adding the equation). Briefly, the spore of *A. niger* diluted to the test concentration by optical density (at final concentration 6 log CFU/mL approximately) and separated into several flasks. The lemongrass oil and ethanol used alone or in combination at MFCs were added to each flask, except to the control and incubated at 30°C. During incubation time, 15 mL sample was removed from the flasks at 0, 2, 4, 6, 8, 19, 12, 14 and 16 hours of incubation. The samples were immediately filtered using 0.22 µm syringe filters to remove bacteria and recorded by spectrophotometer at 260 nm and by the conductivity meter. The effect of antifungal agents to the leakage of cytoplasmic contents was evaluated by the following equation:

$$\delta_{OD} = OD_t - OD_0$$

$\delta_{OD}$ : delta values of UV absorption

$OD_t$ : OD value at t time

$OD_0$ : Initial OD value

$$\delta_{conductivity} = C_t - C_0$$

$\delta_{conductivity}$ : delta values of electrical conductivity

$C_t$ : Electrical conductivity value at t time

$C_0$ : Electrical initial conductivity value.

The treated and untreated samples after 8h of incubation were observed through the Scanning Electron Microscope (SEM) to evaluate the effect of antifungal agents to the spore of *A. niger*.

**Statistical analysis**

The data were subjected to analysis of variance (ANOVA) using SigmaPlot 11 followed by Student-Newman-Keuls t-test to compare means, with a significance

level of 5% when the significant difference between treatments was noted. All tests were performed in triplicate and the data expressed as means ± standard deviation.

**RESULTS**

**The chemical composition of the lemongrass oil**

Major components of lemongrass oil were confirmed and listed in Table 1.  $\beta$ -citral was identified as the main compound with the highest peak area percentage (41.2%).  $\alpha$ -citral (39.80%) was the second major compound detected in the lemongrass oil, followed by Neryl acetate (8.1%); Caryophyllene (1.5%), Linalool (1.5%), Caryophyllene oxide (1.1%). Other compounds such as Verbenol, Carveol, Eucalyptol ... were found to be at the trace level.

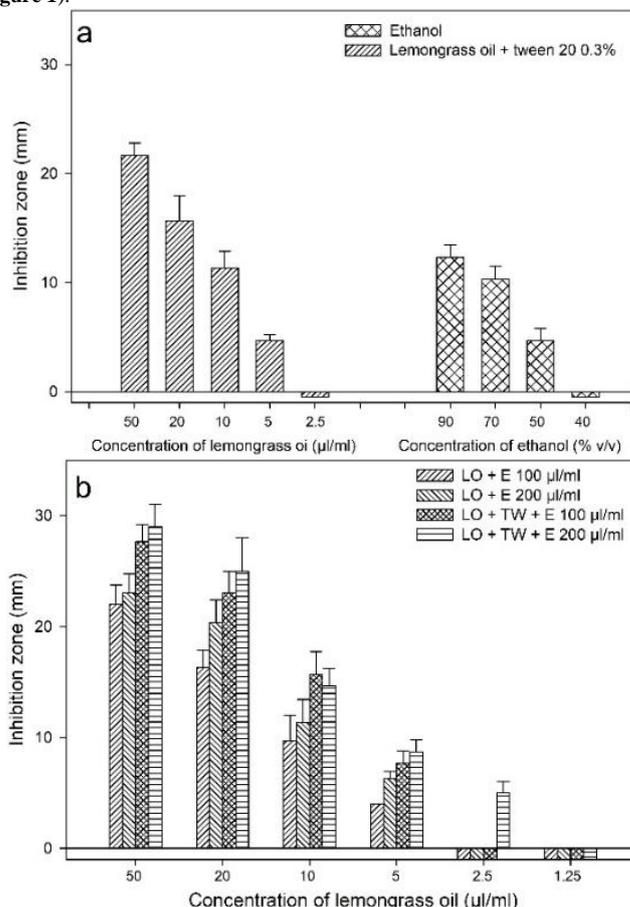
**Table 1** Major components of lemongrass oil

Components	%*
$\beta$ -Citral	41.2
$\alpha$ -Citral	39.8
Camphene	0.3
5-Hepten-2-one, 6-methyl-	1.5
Limonene	0.2
Eucalyptol	0.5
Linalool	1.5
Citronellal	0.3
Verbenol	0.6
Carveol	1.0
Neryl acetate	8.1
Caryophyllene	1.5
Caryophyllene oxide	1.1

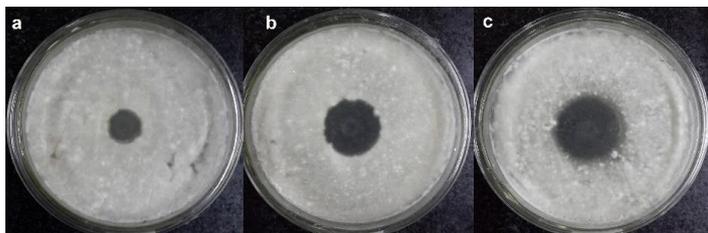
\*Percent of the peak area of the evaporated organic compound

**Antifungal activity of essential oils and ethanol in the agar diffusion method**

The antifungal activity of lemongrass oil and ethanol were shown in Figure 1 and 2. The antifungal zone depends on the concentration of antifungal agents and their combination. The diameter of the antifungal zone of lemongrass oil, ethanol, and their combination was 4.67±21 mm; 4.67±10 mm and 4±29 mm respectively (Figure 1).



**Figure 1** The impact of lemongrass oil and ethanol used alone (a) or in combination (b) against *A. niger* (LO: lemongrass oil; E: ethanol; TW: tween 20)

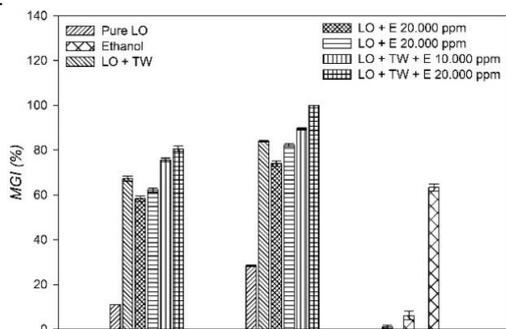


**Figure 2** The diameter of the antifungal zone of ethanol 90% v/v (a), lemongrass oil 50 µL/mL in tween 20 (b) and the combination of lemongrass oil (50 µL/mL) and ethanol (100 µL/mL) in tween 20 (c).

In case of alone treatments, lemongrass oil (in tween 20) showed more antimicrobial effect than ethanol with the minimum inhibitory concentration (MIC) was 5 µL/mL, while the MIC values of ethanol was 500 µL/mL (50% v/v) which was 100 times higher than the lemongrass oil. The result in case of combined treatments showed that the antimicrobial activity of the lemongrass oil in tween 20 was no significant different ( $p>0.05$ ) with the lemongrass oil (without tween 20) in ethanol (Figure 1). The inhibition zone diameter of the combination of lemongrass oil (in tween 20) with ethanol (10%) were higher than these lemongrass oils and ethanol at the same concentration, but the MIC value of the combination of lemongrass oil with ethanol (10%) was not different compared to lemongrass oil that using alone. However, the combination of lemongrass oil in tween 20 with ethanol (20% v/v) show the best result (Figure 1, 2). The MIC value of this combination was at 2.5 µL/mL.

**Effects of lemongrass oil and ethanol on Mycelial Growth**

The effects of lemongrass oil and ethanol on *A. niger* mycelial growth are shown in Figure 3. The results showed that, after five days of incubation, the colony diameter of *A. niger* in control sample was 90 mm while in the treatment samples, the colony diameter of *A. niger* was decreased significantly through the MGI (%) values. In case of alone treatment, the MGIs (%) of ethanol at 10,000; 20,000 and 50,000 ppm were 1.11%; 63.33% and 50.33%. The MGIs (%) of lemongrass oil (in tween 20 0.3% v/v) and the combination of lemongrass oil with ethanol 10,000 ppm or 20,000 ppm at 150 and 300 ppm of concentration were 67.22% and 83.89%; 58.33% and 73.89%; 62.22% and 82.22% respectively (Figure 3).



**Figure 3** Effect of lemongrass oil and ethanol used alone or in combination on mycelial growth of *A. niger* (LO: lemongrass oil; E: ethanol; TW: tween 20) The combination of lemongrass oil (in tween 20 0.3% v/v) with ethanol 20,000 ppm showed the best result that the MGI (%) was 100%. The results showed that the MGIs (%) of lemongrass oil were significantly higher than ethanol. The combination of lemongrass oil (in tween 20) with ethanol is necessary to enhance the antifungal activity.

**Antifungal activity of essential oils in the liquid phase**

According to the results obtained (Table 2), the MFC values of lemongrass oil and ethanol has the same result as that observed in the agar diffusion test. In case of alone treatments, the MFC values of lemongrass oil (in tween 20 0.3% v/v) was 250 ppm which has 200 times lower than ethanol that needs to increase the concentration to 50,000 ppm to reach the same result (Table 2).

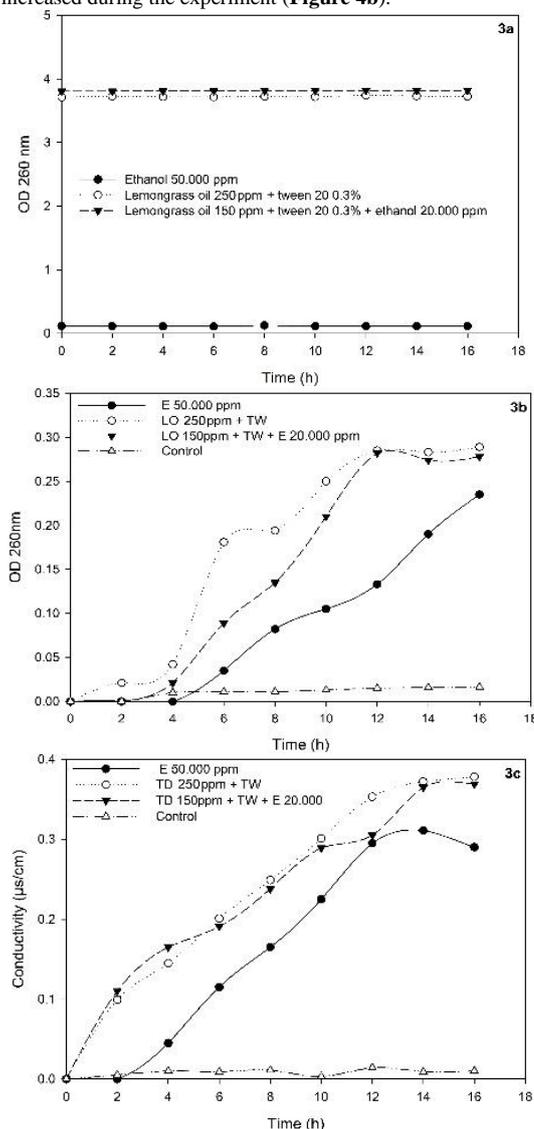
**Table 2** MFCs value of lemongrass oil and ethanol

Antifungal agents	MFC (ppm)
Lemongrass oil	450
Ethanol	50,000
Lemongrass oil + ethanol 1%	280
Lemongrass oil + ethanol 2%	250
Lemongrass oil + tween 0.3%	250
Lemongrass oil + tween 0.3% + ethanol 1%	180
Lemongrass oil + tween 0.3% + ethanol 2%	150

The pure lemongrass oil, which uses alone without tween 20 or ethanol has the lowest antifungal activity with the MFC value was 450 ppm. In case of combined treatments showed that the MFCs values of lemongrass oil (in tween 20 0.3% v/v) with ethanol (10,000 ppm or 20,000 ppm) were 180 ppm and 150 ppm respectively which was significantly lower than in case of alone treatments. The results showed that ethanol and tween 20 enhanced the antifungal activity of lemongrass oil at low concentration in which the combination of lemongrass oil (in tween 20) with ethanol showed the best results.

**The effect of lemongrass oil and ethanol on bacterial cell membrane integrity**

The UV absorption and conductivity of *A. niger* culture supernatants are shown in Figure 4. The results obtained indicate that the UV absorption and electrical conductivity values increased quickly after 16h incubated in the antimicrobial agents (Figure 4). The delta values of UV absorption and electrical conductivity of lemongrass oil 250 ppm (in tween 20); ethanol 50,000 ppm; the combination of lemongrass oil (in tween 20) 150 ppm with ethanol 20,000 ppm were 0.289 and 0.378; 0.235 and 0.290; 0.278 and 0.369 respectively, at 16h incubation and both absorption and conductivity were stable thereafter (Figure 4a; 4c). The delta values of UV absorption and electrical conductivity of the control group were not increased during the experiment (Figure 4b).



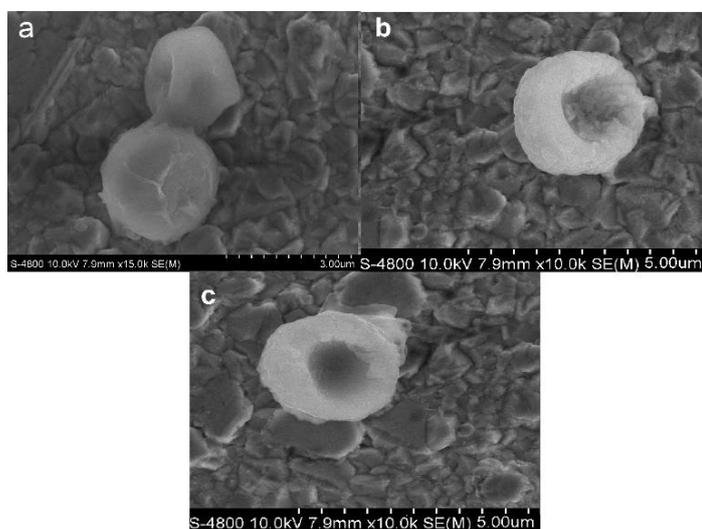
**Figure 4** Effects of lemongrass and ethanol on UV absorption at 260 nm (a and b) and electrical conductivity (c) of *A. niger*; a. the antifungal agents without *A. niger*; b and c. the antifungal agents with *A. niger*

The morphological changes of the *A. niger* were observed through SEM to evaluate the effect of the antifungal agents on the *A. niger*. Figure 5b, 5c shows that the morphological changes of the *A. niger* spores due to significant wrinkles and distortion in the samples treated by lemongrass oil (in tween 20) or lemongrass oil (in tween 20) combining with ethanol while the *A. niger* spores in ethanol show slight wrinkles (Figure 5a). The results showed that, after 8h incubation, the morphology of the *A. niger* spores in the lemongrass oil (250 ppm) in tween 20 sample was wrinkles and distortion (Figure 5b). The similar

result also observed in the lemongrass oil (150 ppm) in tween 20 combined with ethanol (20% v/v) (Figure 5c).

## DISCUSSION

Control of *A. niger* during the preservation is necessary because of their effect caused to post-harvest losses (Prakash *et al.*, 1988; Lieu *et al.*, 2018b). Plain essential oils are gaining interest in their antimicrobial activity. Due to the components of essential oil depending on the variety of plant, the part of the plant considered, harvesting seasons, storage conditions, the concentration of essential oil... as well as the type of tested microorganism (Burt *et al.*, 2004; Tajkarimi *et al.*, 2010, Tyagi *et al.*, 2010; Lieu *et al.*, 2018a), which make it difficult to compare results across laboratories. It is well-known that essential oil affects bacterial cells by many different mechanisms such as the following: disrupt the phospholipid bilayer of the cell membrane; break down the phospholipid membrane which leading to structural breaking, affecting the integrity of cell membranes and changing the permeability of H<sup>+</sup> and K<sup>+</sup> ions (Dinesh *et al.*, 2013; Dharini *et al.*, 2014). The lemongrass oil with monoterpenes is formed by  $\alpha$ -citral and  $\beta$ -citral that the main antimicrobial activity, which showed an inhibitory effect and causing distortion of cytoplasmic membranes of *Candida albicans* (Tyagi *et al.*, 2010). Similarly, the antimicrobial action of ethanol, which able to penetrate the cell wall, causing protein degradation, lipid dissolution, and finally cellular breakage (Weber *et al.*, 1996).



**Figure 5** Scanning Electron Microscope of *A. niger* spores treated with lemongrass and ethanol. **a.** *A. niger* spores were grown in the presence of ethanol 50,000 ppm; **b.** *A. niger* spores grown in the presence of lemongrass oil (in tween 20) 250 ppm; **c.** *A. niger* spores grown in the presence of the combination of lemongrass oil (in tween 20) 150 ppm and ethanol 20,000 ppm.

In previous studies, ethanol (20% v/v) showed the ability to reduce the spore growth of *Botrytis cinerea* compared to the control (Ozgun *et al.*, 2005) as well as to reduce the grape damage caused by *Botrytis cinerea*, while the combination of ethanol and chitosan is necessary to control fungi effectively (Gianfranco *et al.*, 2007). In the present study indicates that ethanol shows the antifungal activity against *A. niger* at 500  $\mu$ L/mL in the agar diffusion test and 50,000 ppm in MFC test which has 100 times and 200 times higher than lemongrass oil (5  $\mu$ L/mL and 250 ppm respectively) (Figure 1 and Table 2). These suggest that lemongrass oil was more effective at inhibiting *A. niger* than ethanol. The differences in the antimicrobial concentration of essential oil in agar diffusion tests and broth dilution assays were reported in previous studies (Boubaker *et al.*, 2016; Lieu *et al.*, 2018a). The differences are due to the type of media, the ability to dilute the essential oil of emulsifiers. Verica *et al.* (2014) reported that the agar diffusion method is not considered an ideal method for essential oil dilution, because of their volatile and poorly soluble components. This makes the essential oil in the agar diffusion method is required high concentration than broth dilution method to have the equivalent antimicrobial effect (Figure 1 and Table 2). In the MFC assays, pure lemongrass oil showed the lowest antifungal activity (Table 2). Water is not an effective method for dispersing the essential oil due to the insoluble phenolic compounds, leading to a decrease in the antimicrobial activity of essential oil (Laird, 2011). Therefore, the emulsifying agent is necessary to enhance antifungal activity.

Due to hydrophobic properties, high volatility and flavoring properties of essential oil which could affect the organoleptic quality of food, the essential oil needs to dilute to required concentrations to ensure sufficient antimicrobial effect without affecting the organoleptic properties of the food. In previous studies, the essential oil was usually diluted in emulsifier such as tween 80, tween 20, xanthan gum (Boubaker *et al.*, 2016; Lieu *et al.*, 2018a,b) or other antimicrobial agents (Ghellai *et al.*, 2015) to enhance the solubility of essential oil and reduce

the amount of essential oil needed to use. The combination of essential oil 0.5% (v/v) with acetic acid 0.25% (v/v) and lactic acid 0.25% (v/v) showed the antimicrobial effect was equivalent to essential oil 1% (v/v) (Ghellai *et al.*, 2015). However, the combination of essential oil (in the emulsifying agent) and ethanol is poorly reported. The solubility of lemongrass in tween 20 is better than ethanol (data not showed). The antifungal activity of the combination of lemongrass oil (in tween 20) and ethanol was improved significantly (Figure 1, 2, 3, 4). This finding suggests that ethanol has a synergistic effect which enhances the antimicrobial activity of lemongrass oil. Meanwhile, tween 20 helps to disperse the essential oil in the water phase and increasing the antimicrobial activity. The combination of lemongrass oil and ethanol showed the antimicrobial activity increase when increasing the ethanol concentration (over 20% v/v) (data not showed). However, the use of ethanol at high concentration in agricultural product preservation would raise costs and the risk of un-safety (Ozgun *et al.*, 2005). In the present study, the combination of lemongrass oil, tween 20 (0.3% v/v) and ethanol showed the best result which not only minimizes the amount of essential oil but also ensures the antimicrobial activity.

The use of UV absorption assay, an electrical conductivity test, and SEM observation are considered an effective way to evaluate the antifungal effect of antifungal agents. In the previous study, the SEM results showed that, at a concentration of 0.5  $\mu$ L/mL, lemongrass caused swelling of the cell wall and much of the cell contents in many bacteria were lost when increasing to 2  $\mu$ L/mL (Jareerat *et al.*, 2011). The antimicrobial components of lemongrass oil can cause yeast deformation or bacterial membrane deformation, leading to leakage of cellular contents (Tyagi *et al.*, 2010; Jareerat *et al.*, 2011). In the present study, the initial absorbance values and electrical conductivity of the samples treated with antimicrobial agents are much different, this is due to the antimicrobial agents caused different absorbance. However, the Figure 4 shows that the UV absorption values and electrical conductivity values in the samples treated by antimicrobial agents that without *A. niger* were almost unchanged at the point of time (0h, 2h, 4h, 6h, 8h, 10h, 12h, 14h, and 16h). These results indicated that the concentration of antimicrobial agents does not affect the changes in absorbance values and electrical conductivity of the culture medium during the experiment. It is interesting to note that, the delta value and SEM result in the combination of lemongrass oil (150 ppm) in tween 20 0.3% v/v and ethanol 20,000 ppm were no significant difference compared to lemongrass oil 250 ppm in tween 20 (0.3% v/v) which 40% higher than that lemongrass oil in the combination treatment. These data suggest that ethanol has a synergistic effect which enhances the antifungal activity of lemongrass oil. This combination is necessary to ensure the antimicrobial effect as well as reduce the amount of used essential oil.

## CONCLUSION

The results indicated that the MIC and MFC values of lemongrass oil (in tween 20 0.3% v/v) were 5  $\mu$ L/mL and 250 ppm respectively that 100 and 200 times lower than ethanol. The antifungal effect of the combination of ethanol and lemongrass oil against *A. niger* showed better results than used alone. The combination of lemongrass oil (in tween 20) and ethanol (20% v/v) with the inhibition zone and MGI (%) value was at 2.5  $\mu$ L/mL and 100% respectively that showed the best result. The UV absorption and electrical conductivity values increased quickly during incubated in the antifungal agents, whereas there were almost unchanged in the control samples at the point of time. The SEM results show that the morphological changes of the *A. niger* spores due to ethanol (50,000 ppm) treatment showed slight wrinkles whereas, in the samples treated by lemongrass oil or lemongrass oil combining with ethanol, *A. niger* spores were significant wrinkles and distortion. The results showed that ethanol and tween 20 enhanced the antifungal activity of lemongrass oil at low concentration in which the combination of lemongrass oil (in tween 20) and ethanol showed the best results. This combination is necessary to ensure the antimicrobial effect as well as reduce the amount of used essential oil.

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