Efficacy of Edible Film Incorporated with Essential Oils against White-Rot Decay Fungus (*Trametes versicolor*)

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Abstract—Antifungal activities of edible film incorporated with essential oils (cinnamon oil, clove oil, anise oil, citronella oil, orange oil, tangerine oil, turmeric oil, guava leave oil, nutmeg oil and lime oil) against a white-rot decay fungus (*Trametes versicolor*) identified from rubberwood were investigated. The disc dilution method was employed to determine the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) by mixing edible film with essential oil at ratios 1:1, 1:2 and 1:4. It was found that cinnamon oil, clove oil and anise oil were the strongest inhibitors with the MICs and MFCs of 1:4. Decay fungi test of *T. versicolor* on treated rubberwood (coated with edible film incorporated with essential oils at ratio 1:4) were then conducted. After 12 weeks of exposure at 25°C and 100%RH, the average weight loss of each rubberwood sample was determined. The results indicated that rubberwood treated with edible film incorporated with cinnamon oil, clove oil and anise oil at ratio 1:4 were classified as “highly resistant” with weight losses of $\leq 10\%$. These findings suggested that edible film incorporated with essential oil has good potential for protecting rubberwood products from the attack of *T. versicolor*.

Index Terms—Antifungal activity, decay fungi, edible film, essential oil.

I. INTRODUCTION

Rubberwood (*Hevea brasiliensis*) lumber obtained from cut down 25-30 years old plantation-grown rubber trees [1] has long been used in furniture industries where rubberwood lumber is generally impregnated with boron preservatives to protect against insect borers and decay fungi [2]. An attempt to utilize the lumber to other applications that are more health-concerned, such as food packaging, kitchenware, children's toys, and indoor wooden structures has recently been explored [3]. Instead of using synthetic chemicals to provide a protection on wood, biological control of utilizing essential oil extracted from herbs or plants [4] to suppress mold on surface [3], [5], to inhibit the attack of the decay fungi [5], [6] and to protect against termite [7] has proved to be a powerful alternative.

Antifungal effects against molds of essential oils extracted from anise oil, lime oil, tangerine oil [3], cinnamon [5], and

lemongrass, rosemary, tea tree, thyme [8], cinnamon [5] have been reported. Essential oils extracted from *Calocedrus formosana* leaf [6], cassia oil and wood tar oil [7] have been reported to resist the attack of both wood decay fungi and termite. So far there has been no report on antifungal effects of wood coated with edible film incorporated with essential oils against decay fungi. The present work deals with the inhibitory effects of rubberwood coated with edible film incorporated with cinnamon oil, clove oil, anise oil, citronella oil, orange oil, tangerine oil, turmeric oil, guava leave oil, nutmeg oil and lime oil against a white-rot decay fungus (*Trametes versicolor*) commonly found on rubberwood.

II. MATERIALS AND METHODS

A. Essential Oils

Cinnamon oil (containing 74% cinnamaldehyde), clove oil (containing 75% eugenol), anise oil (containing 95% trans-anethole), citronella oil (containing 86% d-limonene), orange oil (containing 62% d-limonene), tangerine oil (containing 78% d-limonene), turmeric oil, (containing 23% phellandrene), guava leaf oil, nutmeg oil (containing 35% sabinene), and lime oil (containing 79% d-limonene), were purchased from Thai China Flavours & Fragrances Industry Co., Ltd. Bangkok, Thailand.

B. Preparation of Inoculums

A decay white-rot fungus (*Trametes versicolor* WU 0704) was identified from rubberwood. Codes refer to strains held in the culture collection of the Wood Science and Engineering Research Unit, Center for Scientific and Technological Equipments, Walailak University, Nakhon Si Thammarat province, Thailand.

C. Determination of the Minimal Inhibitory Concentration (MIC) and the Minimal Fungicidal Concentration (MFC)

The MIC of the test on the decay fungus was determined using an disc dilution method by preparing edible film from whey protein incorporated with cinnamon oil, clove oil, anise oil, citronella oil, orange oil, tangerine oil, turmeric oil, guava leave oil, nutmeg oil and lime oil at ratios 1:1, 1:2 and 1:4. The components were mixed until homogeneous by homogenizer. Then, the whey protein films were formed with the method described by Matan [9]. The film forming solutions (27.5 ml) were cast onto 9 cm diameter circular Teflon® surfaces and then dried overnight at 35°C and $45 \pm 5\%$ RH. The dried weight of the film was 7.15 grams. Dried films were peeled and stored at $40 \pm 5\%$ RH. Film thickness was measured with a digital micrometer (Digimatic Micrometer, Mitutoyo, Japan) to the nearest 0.0001 mm. Measurements were taken at twelve random locations of each film sheet.

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Average film thickness was 0.2412 mm. Circular “discs” were cut from the edible films using a cutting well. The vegetable oil was used as a control. The fungus was inoculated on the medium. An edible film disc (9 cm in diameter) was placed on the top of each Petri dish. The Petri dish was then incubated at 25°C. Tests were performed in triplicate. The lowest concentration showing no visible growth was regarded as the MIC.

A disc of mycelial material from the plate showing no growth were subcultured on malt extract agar plates to determine if the inhibition was reversible or permanent. The MFC was determined as the lowest concentration at which no growth occurred on the plates after subculturing.

D. Decay Fungi Test on Rubberwood

Sets of five random replicate rubberwood specimens (25mm×25mm×25mm) were vacuum-treated according to the ASTM test method D1413-05b [10] with individual edible film incorporated with cinnamon oil, clove oil, anise oil at ratios 1:4. Five control blocks were treated with pure vegetable oil. The vacuum treatment schedule included 30 min vacuum at 100 mmHg. After vacuum release, specimens were left to spray by edible film incorporated with those oils at ratio 1:4. Test blocks were conditioned to ensure all solvent had dissipated prior to being placed half buried into glass bottles with sterilized substrate and distilled water. Sterilized blocks were set in the bottles, inoculated with the white-rot decay fungus, and then incubated at 25°C, 100%RH for 12 weeks. The percentage weight losses were calculated in the individual test block from the conditioned weights before and after exposure to the decay fungi.

E. Statistical Analyses

The data was statistically treated by one-way ANOVA and Duncan's post hoc test with $P < 0.05$ was considered to be statistically significant.

III. RESULTS AND DISCUSSION

A. Inhibition of a White Rot Decay Fungus

The inhibitory effects of edible film incorporated with cinnamon oil, clove oil, anise oil, citronella oil, orange oil, tangerine oil, turmeric oil, guava leave oil, nutmeg oil and lime oil on the white-rot decay fungus are shown in Table I. It is evident that edible film incorporated with cinnamon oil (Fig. 1), clove oil and anise oil at ratio 1:4, exhibited both fungistatic and fungicidal activities against the test.

Cinnamon oil and clove oil, in particular, are both natural preservative and flavoring substances that are not harmful when consumed in food products [11]. There have been a number of reports of substances in each of cinnamon and clove oils that inhibit the growth of molds, yeasts and bacteria. Both cinnamon oil and clove oil added at 2% in potato dextrose agar (PDA) completely inhibited the growth of seven mycotoxigenic moulds (*Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, *Penicillium* sp. M46, *P. roqueforti*, *P. patulum*, and *P. citrinum*) for various times up to 21 days [12] and could also inhibit the growth of yeasts [14]. In addition, Soliman and Badeaa [13] found that ≤ 500 ppm of cinnamon oil can inhibit *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme* on PDA. August (1978)

[15] reported that high concentrations of cinnamon oil and clove oil could also inhibit the asexual spores of fungi. It was reported that cinnamaldehyde, major component of cinnamon oil, and eugenol, major component of clove oil, possessed the antifungal activities against wood decay fungi [15]

TABLE I: MINIMAL INHIBITORY CONCENTRATIONS (MIC) AND MINIMAL FUNGICIDAL CONCENTRATIONS (MFC) OF ESSENTIAL OIL AGAINST *TRAMETES VERSICOLOR*

Essential oil	MIC		
	Edible film : Essential oil		
	1:1	1:2	1:4
Cinnamon oil	+	-	-
Clove oil	+	-	-
Anise oil	+	-	-
Citronella oil	+	+	-
Orange oil	+	+	-
Tangerine oil	+	+	+
Turmeric oil	+	+	-
Guava	+	+	-
Nutmeg oil	+	+	+
Lime oil	+	+	+
Control	+	+	+
Essential oil	MFC		
	Edible film : Essential oil		
	1:1	1:2	1:4
Cinnamon oil	+	+	-
Clove oil	+	+	-
Anise oil	+	+	-
Citronella oil	+	+	+
Orange oil	+	+	+
Tangerine oil	+	+	+
Turmeric oil	+	+	+
Guava	+	+	+
Nutmeg oil	+	+	+
Lime oil	+	+	+
Control	+	+	+

+ =Growth

- = No-growth

Fig. 1. Growth of *Trametes versicolor* on edible film incorporated with vegetable oil (A) and with cinnamon oil at ratio 1:4 (B) after 3 day at 25°C.

B. Decay Test on Rubberwood

The results of the decay test on rubberwood have been summarised in Fig. 2. Statistical analysis of this data showed significant differences between weight losses of the control and the treated specimens as a result of the attack from *Trametes versicolor*, indicating that edible film incorporated

with cinnamon oil, clove oil and anise oil did influence decay resistance of rubberwood. Moreover, edible film incorporated with cinnamon oil, clove oil and anise oil at ratio 1:4 reduced weight loss of rubberwood after 12 weeks to about a one-fifth of the control treated with vegetable oil and were classified as “highly resistant” (weight losses $\leq 10\%$).

Cinnamon oil and clove oil, in particular, are both natural preservative and flavoring substances that are not harmful when consumed in food products. It was reported that cinnamaldehyde, major component of cinnamon oil, and eugenol, major component of clove oil, possessed the synergistic effects against wood decay fungi [2].

The effectiveness of anise oil as an antifungal agent was also reported in 2001[16]. Main component of anise oil (trans-anethole), is believed to be the main inhibitory component [17].

Fig. 2. Percentage weight losses of rubberwood specimens exposed to *Trametes versicolor* for 12 weeks ^{a,b} Mean value by the same letter are not significantly ($P < 0.05$) different according to Duncan test following ANOVA.

IV. CONCLUSIONS

Edible film incorporated with cinnamon oil, clove oil and anise oil at ratio 1:4 exhibited both fungistatic and fungicidal activities against the white-rot fungus *Trametes versicolor* identified from rubberwood on agar medium. In addition, rubberwood coated with edible film incorporated with those oils showed highly resistance to fungal decay caused by *Trametes versicolor*.

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