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BIOCONTROL OF *Penicillium chrysogenum* USING NUTMEG OIL AND TURMERIC OIL

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ABSTRACT

Antifungal activities of nutmeg oil and turmeric oil against *Penicillium chrysogenum* screening from rubberwood kitchenette surface were investigated. The broth dilution method was employed to determine the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) using the concentration of essential oils between 10-250 µL/mL. Inhibitory effects of the essential oils against mould on rubberwood were also examined by means of the dip treatment. It was found that the MIC and MFC values for each treatment were identical for all conditions examined. The MIC and MFC of nutmeg oil and turmeric oil against *Penicillium chrysogenum* were 100 µL/mL and 200 µL/mL, respcetively. In addition, nutmeg oil and tumeric oil at MIC and MFC provided a protection from mold growth on rubberwood for at least 8 weeks at the storage condition of 30°C with 100%RH.

KEYWORDS: biocontrol, *Penicillium chrysogenum*, nutmeg oil, turmeric oil

1. INTRODUCTION

The growing demand for healthy wood products is in line with the increased utilization of wood chemical such as boron agents to ensure an efficient and rigorous control of their deterioration [1-2]. Boron and/or chemical preservatives have proven to resist mould on the surface, and are used in food packaging, kitchenware, children's been toys and indoor wooden structures. In addition, the growing consumer awareness and/or demand for healthy products increased the quest for more efficient methods for the control of diseases with minimum health and environmental impact [3]. On surface of wood, an alternative to the use of synthetic chemicals are demanded [4]. Essential oils and their constituents have a long history of applications as antimicrobial agents. Production of essential oils by plants is believed to be predominantly a defence mechanism against microorganism [5] and indeed, essential oils have been shown to possess antimicrobial and antifungicidal properties [6-8]. Essential oils and their components are gaining increasing interest because of their relatively safe status and their wide acceptance by consumers [9]. The present work deals with the inhibitory effects of nutmeg oil and turmeric oil on growth of *Penicillium chrysogenum* commonly found on rubberwood surface. Dip treatment was employed for mould test on rubberwood.

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2. MATERIALS AND METHODS

2.1 Essential oils

Nutmeg oil and turmeric oil derived by steam distillation were provided by Thai China Flavours & Fragrances Industry Co., LTD. Bangkok, Thailand.

2.2 Culture

Mold from rubberwood kitchenette surface was identified as *Penicillium chrysogenum* WU 0702. This mold caused discoloration on 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.

2.3 Preparation of inoculum

Spores were obtained from growing *P. chrysogenum* WU 0702 on malt extract agar (MEA; Merck Ltd, Thailand) medium at 30°C for 14 days and were collected by flooding the surface of the agar plates with ~5 ml sterile saline solution (NaCl, 8.5 g/l water) containing Tween 80 (0.1% v/v). After counting the spores using a haemocytometer, the suspension was standardized to concentrations of 10^7 spore/ml by dilution with sterile water before using. The viability of all strains checked using quantitative colony counts were at 10^7 CFU/ml.

2.4 Inhibition of Penicillium chrysogenum WU 0702 by essential oils

Determinations of a minimal inhibitory concentration (MIC) and a minimal fungicidal concentration (MFC) of the oils were performed by broth dilution method in test tubes. The essential oil of 50 µl from each of 10 to 250 µL/mL was added to 5 ml of yeast extract sucrose broth (YES) tubes containing 10^7 spores/ml. The vegetable oil was used as a control. Different dilutions of the oils (including controls) were made with methanol. The preliminary study revealed that methanol had no effect on mold growth. The tubes were then incubated at 30°C for 3 days on an incubator shaker (Gallenkamp, Loughborough, England) as to evenly disperse the oil throughout the broth . The highest dilution (lowest concentration), showing no visible growth, was regarded as the MIC. Cells from the tubes showing no growth were subcultured on potato dextrose agar plates to determine if the inhibition was reversible or permanent. MFC was determined as the highest dilution (lowest concentration) at which no growth occurred on the agar plates [10].

2.5 Dip treatment of rubberwood

Rubberwood specimens (7mm x 20mm cross section by 70 mm long) were prepared from freshly cut rubberwood lumber obtained from the plantation site in Nakhon Si Thammarat province, Thailand. The average moisture content of the rubberwood specimens before testing was 49±2 % (n=10). Sets of five random replicate specimens were dip treated according to ASTM test methods D4445-91 [11] for 15 seconds with nutmeg oil at 100 µL/mL and turmeric oil at 200 µL/mL. Vegetable oil was used as a control. Different dilutions of the oils were made with methanol. A dip treated and vacuum treated specimens was held in a closed container overnight at room temperature before inoculation with spores of the test mold. Specimens were weighed before and after the treatment to estimate the retention levels of essential oil in wood specimens. The oil treated specimens were inoculated with 1 mL of each of mold-spore inoculum (10^7 spores/ml) and were incubated at 30°C with 100% RH in an environmental chamber (Contherm, Petone, New Zealand) for 12 weeks. The specimens were then individually rated for mold growth on a scale of 0 to 5, with 0 meaning clean specimens and 5 meaning heavy mold growth (0=clean, 1=20%, 2=40%, 3=60%, 4=80%, 5=100% of mold growth) according to ASTM test methods D4445-91. Percent of stain and mold (based on control) for each essential oil concentration was calculated

according to Percent of stain and mold (based on control) = (A/B) x100 (When A =Total score for each mold at each concentration of essential oil; B = Total score for each mold at control).

3. RESULTS AND DISCUSSION

Fungal growth inhibition was observed for both essential oils (Table 1). MIC and MFC techniques were employed to assess fungistatic and fungicidal properties of the oils. It was found that nutmeg oil and turmeric oil had static and fungicidal effect at 100 µL/mL and 200 µL/mL, respectively.

Table1 Minimum inhibitory concentration and minimum fungicidal concentration of the essential oil against *P. chrysogenum*

Essential oil	MIC	MFC
Nutmeg oil	100	100
Turmeric oil	200	200

Essential oils derived from many plants are known to possess antifungal activities [12]. Essential oils extracted from spices and herbs are generally recognized as containing active antimicrobial compounds [5]. Borneol, geraniol, linalool, terpineol, eugenol, myristicin, safrole, camphene, dipentene and pinene are components in nugmet oil that inhibit the growth of both bacteria and mold [13]. Nutmeg are very popular spices and commonly added to sausage and some traditional foods. Curcumin is active compounds in turmeric oil and possesses antimicrobial activity. In addition, protective effects of turmeric against aflatoxin induced liver damage has been reported in chickens [14]. Mold resistance of dip treated inoculated with *P. chrysogenum* are shown in Figure 1. The results are presented as the average ratings of five specimens. Specimens were initially rated after being incubated for 1 week. Ratings continued periodically through 12 weeks or until test oils failed to substantially inhibit test fungi. Results of the dip method showed that rubberwood treated with nutmeg oil was about 40% covered with mold growth at week 9 and 100% covered at week 10 whereas turmeric oil inhibited *P. chrysogenum* WU02 for up to 8 weeks and showed 100% mould coverage at week 10. Oil treatment may have some effects on moisture exclusion, but control stakes dipped with vegetable oil showed 100% mold coverage at week 2. From the result, nutmeg oil and turmeric oil were effective in inhibiting mold growth on rubberwood. Leaching characteristics of essential oil components from rubberwood under various conditions is another key area that should be explored in the future.

4. CONCLUSIONS

Nutmeg oil and turmeric oil were effective against *P.chrysogenum* on rubberwood dip treated with the oils at certain period. Both of oils provided protection from mold growth on rubberwood for at least 8 weeks at 100 µL/mL of nutmeg oil and 200 µL/mL of turmeric oil. These antifungal compound may be useful for mould inhibition on rubberwood. Other application of essential oils on food packaging, kitchenware can be utilized.

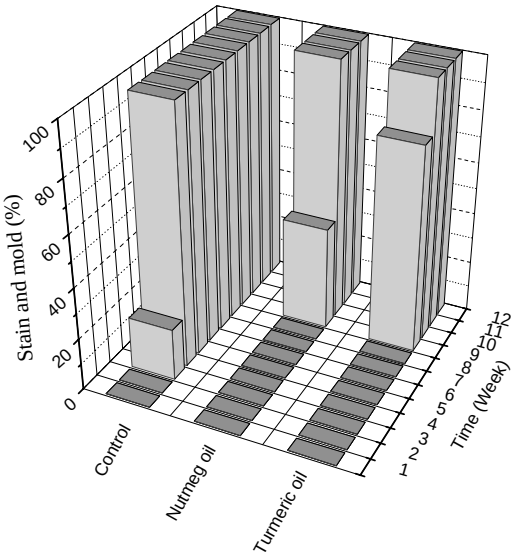

Figure 1 Percent of stain and mold (based on control at 100 µL/mL of nutmeg oil and 200 µL/mL of turmeric oil)

5. ACKNOWLEDGEMENTS

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