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# INHIBITORY EFFECT OF ESSENTIAL OILS ON THE GROWTH OF *Aspergillus flavus*

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## ABSTRACT

The effects of 16 essential oils from aromatic plants were tested for their inhibitory effect on *Aspergillus flavus* IMI 242684 on PDA. The results showed that the essential oil of white wood (*Melaleuca cajeputi*) gave the highest inhibition followed by the essential oils of cinnamon (*Cinnamomum cassia*) and lavender (*Lavandula officinalis*), respectively. Furthermore, the inhibitory effects of these three essential oils at different concentrations were examined. It was found that the essential oil of white wood at 1.5625% (v/v) and of cinnamon and lavender at 50% (v/v) were the optimum concentrations for fungal growth inhibition. The essential oil of white wood at 25% (v/v) completely inhibited the growth of *A. flavus* IMI 242684 on PDA for 28 days.

**KEYWORDS**: essential oils, aromatic plants, aflatoxin, *Aspergillus flavus*

## 1. INTRODUCTION

Aflatoxins are secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus*, *A. nomius*, *A. tamarii* and *A. bombycis* [1-3]. These toxins are acutely and chronically toxic to both humans and animals [4] and they are among the most potent mutagenic and carcinogenic compounds known to be produced in nature [5]. Aflatoxins have also been identified as a potential biological weapon for food and water contamination [6]. Various agricultural commodities have been found to be contaminated with either aflatoxin producing fungi or aflatoxins [7]. Fermented foods and beverages in Thailand such as fermented rice (kaomak), soybean sauce (taotjo), peanut butter, soy sauce (shoyu), Thai red and white wine and rice sugar wine were contaminated with aflatoxin producing fungi [8]. Aflatoxin contamination was shown to be particularly high for low-grade chilies and chili powder sold in supermarkets in India and corn and groundnut samples employed in the preparation of poultry feed [7]. Occurrences of aflatoxin in medicinal plants and spices have also been established [9-10].

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Natural plant extracts are of interest as a source of safer or more effective substitutes for synthetically produced antimicrobial agents and may provide an alternative way to prevent food or feed from fungal contamination [11-14]. Powders and extracts of various herbs, spices and essential oils have been reported to have antimicrobial activity and some also to inhibit aflatoxin formation [15-22]. The present study was undertaken to examine the inhibitory effect of essential oils from 16 plants on the growth of *A. flavus* IMI 242684 on PDA.

## 2. MATERIALS AND METHODS

### 2.1 Medicinal plants

Sixteen medicinal plants used in this study were as follows: safflower (*Carthamus tinctorius*)**,** marigold (*Tagetes erecta*), coriander (*Coriandrum sativum*), pomelo (*Citrus maxima*), mangosteen (*Garcinia mangostana*), krachaidum (*Kaempferia parviflora*), ginger (*Zingiber officinale*), pepper (*Piper nigrum*), Boraphet (*Tinospora crispa*), aloe (*Aloe vera*), lavender (*Lavendula officinalis*), rosemary (*Rosemarinus officinalis*), cinnamon (*Cinnamomum cassia*), eucalyptus (*Eucalyptus globules*), thyme (*Thymus vulgaris*), and white wood (*Melaleuca cajeputi*).

### 2.2 Preparation of essential oils

Commercial essential oils of lavender, rosemary, cinnamon, eucalyptus, thyme and white wood were purchased from Hong Huad Co. Ltd, Bangkok, Thailand whereas the other plants were bought from local herbal shop in Bangkok and essential oils were extracted by the following procedure.

The dried plant materials were washed and cut into small pieces. One kg of each plant was ground and submerged in ethanol 95% at room temperature for 12 hrs [23]. The extracts were then filtered through cheesecloth. The plant materials were submerged again in ethanol 95% for 3 days and filtered. The filtrates were then collected and evaporated using vacuum rotary evaporator at 50-60$^{o}$ C. The concentrated extracts were dissolved in petroleum ether (AR grade) with boiling point of 40-60$^{o}$ C at room temperature and evaporated using vacuum rotary evaporator at 50-60$^{o}$ C. The concentrated essential oils were then dissolved in ethanol 95% and stored at 4$^{o}$ C for further investigation.

Different parts of the plants used for extraction were as follows: flowers (safflower and marigold); twigs (coriander, Boraphet and aloe); rinds (pomelo and mangosteen); rhizomes (krachaidum and ginger); and raw fruits (pepper).

### 2.3 Fungal strain

*Aspergillus flavus* IMI 242684 obtained from the International Mycological Institute, England was used throughout this study. The culture was maintained in PDA slant agar.

### 2.4 Antifungal assay (Agar diffusion test)

Five ml of potato dextrose agar (PDA) were poured into a Petri dish and left to set and fifteen ml of potato dextrose agar heavily inoculated with spores of *A. flavus* ($10^6$ spores/ml) were poured over the surface of PDA agar plate. Sterile cylinder cups (6mm. dia.) containing 250 µl of each essential oil were placed into the same plates. The plates were then incubated for 3 days at room temperature and the zones of inhibition were determined using vernier digital [24]. All experiments were repeated at least five times

The effective essential oils were then selected and tested for their ability to control the growth of *A. flavus* at concentrations of 1.5625, 3.125, 6.25, 12.5, 25 and 50% (v/v) on PDA for 3 days. The best essential oil was again selected and tested for the inhibitory effect on the growth of *A. flavus* on PDA for 28 days.

### 2.5 Statistical analysis

The experiments were designed by Randomized Complete Block Design and analysed statistically by the analyses of variance (ANOVA) and multiple comparison at the 1% level [25].

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of essential oils on the growth of *A. flavus* IMI 242684 on agar medium

Sixteen essential oils were tested for the effect on the growth of *A. flavus* IMI 242684 on PDA for 3 days. The results show that white wood, cinnamon and lavender significantly inhibited the growth of *A. flavus* more than the other essential oils (Tables 1 and 2). White wood was the most effective oil followed by cinnamon and lavender, respectively.

The effects of white wood, cinnamon and lavender oils at various concentrations (1.5625, 3.125, 6.25, 12.5, 25 and 50%) on the growth of *A. flavus* IMI 242684 on PDA for 3 days were shown in Tables 3 and 4. Statistical analyses showed that white wood at all concentration significantly inhibited the fungal growth whereas cinnamon and lavender inhibited the growth of *A. flavus* at concentrations of 12.5% or higher.

The effect of white wood was further investigated on the inhibitory effect on fungal growth on PDA for 28 days (Tables 5 and 6). The result shows that white wood at concentrations of 25 and 50% completely inhibited the fungal growth for 28 days whereas at concentration of 12.5% completely inhibited the growth of *A. flavus* for only 7 days. White wood at concentrations of 1.5625, 3.125 and 6.25% inhibited the fungal growth with the inhibition zones of 78.38, 80.78 and 84.83 mm. on day 7, respectively.

### 3.2 General discussion

The major constituents of white wood oil are monoterpene compounds such as terpinolene (24.74%) and ϒ-terpinene (22.84% [26]. Mahmoud [27] reported that 100 ppm of five oils, namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde) and thymol (phenolic ketone), completely suppressed growth of *A. flavus* and consequently prevented aflatoxin synthesis in liquid medium. Some of these essential oils could prevent fungal growth and toxin formation for 8 days. The hydrosols of anise, cumin, fennel, mint, picking herb, oregano, savory and thyme showed a strong inhibitory effect on mycelial growth of *A. parasiticus* NRRL 2999 [28].

Thanaboripat *et al*. [20] reported that citronella oil at 0.2% (v/v) inhibited the growth of *A. flavus* IMI 242684, *A. flavus* M113, *A. flavus* S 156 and *A. parasiticus* IMI 102566 on PDA for 21, 7, 7 and 21 days, respectively. Essential oils of cinnamon (*Cinnamomum zeylanicum*), peppermint (*Mentha piperita*), basil (*Ocimum basillicum*), origanum (*Origanum vulgare*), the flavoring herb *epazote* (*Teloxys ambrosioides*), clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) caused a total inhibition of *A. flavus* on maize kernels. The optimum dosage for protection of maize varied from 3 to 8 % [29].

A number of compounds and substances have been found to be effectively inhibit fungal growth and aflatoxin production, while others have stimulatory properties [30]. In many instances low concentrations of test compounds stimulated fungal growth and/or toxin production, while higher concentrations completely inhibited them. Clove oil at 50 and 100 µg/ml and cinnamon oil at 50µg/ml stimulated the growth of *A. flavus* in liquid media whereas higher concentrations reduced the mycelial growth [31]. In the present study, white wood oil exhibited the most

**Table 1** Analysis of variance for sixteen essential oils influencing the growth of *A. flavus* IMI 242684 on PDA for 3 days

| Source of variation | Degree of freedom | Sum of Square | Mean Square | F |
|---|---|---|---|---|
| Essential oils | 4 | 30137.737 | 7534.434 | 64999.483** |
| Error | 20 | 2.318 | 0.166 | |
| Total | 24 | 30140.055 | | |

** = significant difference

**Table 2** Multiple comparison test on the inhibitory effect of sixteen essential oils on the growth of *A. flavus* IMI 242684 on PDA for 3 days

| Type of essential oil | Mean of inhibition zone (mm.)* |
|---|---|
| Control | $0.00^{d}$ |
| White wood | $>90.00^{a}$ |
| Cinnamon | $46.90^{b}$ |
| Lavender | $11.49^{c}$ |
| Other essential oils | $0.00^{d}$ |

*Means in the same column with different superscripts are significantly different.

**Table 3** Analysis of variance for essential oils of white wood, cinnamon and lavender at various concentrations influencing the growth of *A. flavus* IMI 242684 on PDA for 3 days

| Source of variation | Degree of freedom | Sum of Square | Mean Square | F |
|---|---|---|---|---|
| Type of essential oil | 2 | 111490.932 | 55745.466 | 53264.317** |
| Concentration of essential oil | 6 | 21987.255 | 664.542 | 3501.439** |
| Type of essential oil and Concentration of essential oil | 12 | 23899.687 | 991.641 | 1902.996** |
| Error | 84 | 87.913 | 1.047 | |
| Total | 104 | 157465.786 | | |

**Table 4** Multiple comparison test on the inhibitory effect of essential oils of white wood, cinnamon and lavender at various concentrations on the growth of *A. flavus* IMI 242684 on PDA for 3 days

| Concentration of essential oil (%) | Mean of inhibition zone(mm.)* | | |
|---|---|---|---|
| | White wood | Cinnamon | Lavender |
| Control | $0.00^{f}$ | $0.00^{f}$ | $0.00^{f}$ |
| 1.5625 | $>90.00^{a}$ | $0.00^{f}$ | $0.00^{f}$ |
| 3.125 | $>90.00^{a}$ | $0.00^{f}$ | $0.00^{f}$ |
| 6.25 | $>90.00^{a}$ | $0.00^{f}$ | $0.00^{f}$ |
| 12.5 | $>90.00^{a}$ | $20.76^{b}$ | $7.09^{e}$ |
| 25 | $>90.00^{a}$ | $30.71^{c}$ | $7.59^{e}$ |
| 50 | $>90.00^{a}$ | $45.94^{d}$ | $8.69^{e}$ |

*Means with different superscripts are significantly different.

**Table 5** Analysis of variance for essential oil of white wood at various concentrations influencing the growth of *A. flavus* IMI 242684 on PDA for 7, 14, 21 and 28 days

| Source of variation | Degree of freedom | Sum of Square | Mean Square | F |
|---|---|---|---|---|
| Concentration of essential oil | 6 | 114092.407 | 19015.401 | 29129.042** |
| Time (day) | 3 | 37349.255 | 12449.782 | 19017.394** |
| Concentration of essential oil and time | 18 | 31263.281 | 1736.849 | 2660.619** |
| Error | 112 | 73.113 | 0.653 | |
| Total | 139 | 182778.146 | | |

**Table 6** Multiple comparison test on the inhibitory effect of essential oil of white wood at various concentrations on the growth of *A. flavus* IMI 242684 on PDA for 7, 14, 21 and 28 days

| Concentration of essential oil (%) | Mean of inhibition zone (mm.)* | | | |
|---|---|---|---|---|
| | 7 days | 14 days | 21 days | 28 days |
| Control | $0.00^{f}$ | $0.00^{f}$ | $0.00^{f}$ | $0.00^{f}$ |
| 50 | $>90.00^{a}$ | $>90.00^{a}$ | $>90.00^{a}$ | $>90.00^{a}$ |
| 25 | $>90.00^{a}$ | $>90.00^{a}$ | $>90.00^{a}$ | $89.35^{a}$ |
| 12.5 | $>90.00^{a}$ | $88.95^{a}$ | $85.11^{a}$ | $76.38^{b}$ |
| 6.25 | $84.83^{a}$ | $81.27^{a}$ | $74.54^{b}$ | $42.17^{c}$ |
| 3.125 | $80.78^{a}$ | $72.61^{b}$ | $54.97^{c}$ | $8.95^{e}$ |
| 1.5625 | $78.38^{a}$ | $52.06^{c}$ | $29.80^{d}$ | $0.00^{f}$ |

*Means with different superscripts are significantly different.

antimicrobial activity against *A. flavus*. There has been speculation on the contribution of the terpene fraction of the oils to their antimicrobial activity [32]. The antimicrobial activity varies widely, depending on the type of spice or herb, test medium and microorganism [33]. Contents of essential oils in different species is influenced by genetic material, culture conditions, environment and by crop and post-crop processing [34 - 35].

## 4. CONCLUSIONS

Several studies have focused on the potential use of essential oil applications in biological control of aflatoxin producing fungi and insect pests [22, 36]. Certain essential oils can be applied as mold inhibitors in order to prevent the growth of aflatoxigenic fungi in stored food. From this present study, it was found that the essential oil from white wood can be used as mold inhibitor in agricultural products. However, the appropriate application of white wood should further be investigated.

## REFERENCES


[1] Kurtzman, C. P., Horn, B. W. and Hesseltine, C. W. **1987** *Aspergillus nomius*, a new aflatoxin producing species related to *Aspergillus flavus* and *Aspergillus tamari*, *Antonie van Leeuwenhoek*, 53, 147-158.

[2] Goto, T., Ito, Y., Peterson, S.W. and Wicklow, D.T. **1997** Mycotoxin production ability of *Aspergillus tamari*, *Mycotoxins*, 44, 17-20.

[3] Peterson, S.W., Ito, Y., Horn, B.W. and Goto, T. **2001** *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*, *Mycologia*, 93(4), 689-703.

[4] Dvorackova, I. **1990** *Aflatoxins and Human Health*. CRC Press, Inc., Boca Raton, Fla.

[5] Smith, J.E. and Moss, M.O. **1985** *Mycotoxins. Formation, Analysis and Significance*. Chichester., John Wiley & Son.

[6] Smith, J.E. **2004** *Biotechnology*. 4th ed. Cambridge University Press, Cambridge.

[7] Reddy, D.V.R., Thirumala-Devi, K., Reddy, S.V., Waliyar, F., Mayo, M.A., Rama Devi, K., Ortiz, R.and Lenne, J.M. **2002** Estimation of aflatoxin levels in selected foods and feeds in India. In: Hanak, E., Boutrif, E, Fabre, P. and Pineiro, M.,Eds. *Food Safety Management in Developing Countries.* Proceedings of the International Workshop, CIRAD-FAO, Montpellier, France, pp. 1-4.

[8] Sripathomswat, N. and Thasnakorn, P. **1981** Survey of aflatoxin-producing fungi in certain fermented foods and beverages in Thailand, *Mycopathologia,*, 73, 83-88.

[9] Roy, A.K., Sinha, K.K. and Chourasia, H.K. **1988** Aflatoxin contamination of some common drugs, *Applied and Environmental Microbiology*, 54(3), 842-843.

[10] Aziz, N.H., Youssef, Y.A., El-Fouly, M.Z. and Moussa, L.A. **1998** Contamination of some common medicinal plant samples and spice by fungi and their mycotoxins, *Botanica Bulletin Academica Sinica*, 39, 279-285.

[11] Yin, Mei-Chin and Cheng, Wen-Shen. **1998** Inhibition of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices, *Journal of Food Protection*, 61(1),123-125.

[12] Mahmoud, A.-L.E. **1999** Inhibition of growth and aflatoxin biosynthesis of *Aspergillus flavus* by extracts of some Egyptian plants, *Letters in Applied Microbiology*, 29, 334-336.

[13] Thanaboripat, D. **2002** Importance of aflatoxins, *KMITL Science Journal*, 2(1),38-45.

[14] Thanaboripat, D. **2003** Mycotoxins: Occurrence and control in foods. In: *The International Review of Food Science and Technology*. IUFoST, November, 2003, pp. 131-133.

[15] Masood, A., Dogra, J.V.V. and Jha, A.K. **1994** The influence of colouring and pungent agents of red chilli (*Capsicum annum*) on growth and aflatoxin production by *Aspergillus flavus*, *Letters in Applied Microbiology*, 18, 184-186.

[16] Prasad, G., Sahay, S.S. and Masood, A. **1994** Inhibition in aflatoxin biosynthesis by the extract of *Amorphophallus campanulatus* (OL) and calcium oxalate, *Letters in Applied Microbiology*, 18, 203-205.

[17] Thanaboripat, D., Naranong, N. and Peerapakorn, N. **1989** Effect of some herbs on growth of *Aspergillus flavus* and aflatoxin production, *Srinakarinwirot Journal of Science*, 5,33-39.

[18] Thanaboripat, D., Nontabenjawan, K., Leesin, K., Teerapiannont, D., Sukcharoen, O. and Ruangrattanametee, V. **1997** Inhibitory effect of garlic, clove and carrot on growth of *Aspergillus flavus* and aflatoxin production, *Journal of Forestry Research*, 8, 39-42.

[19] Thanaboripat, D., Cheunoy, W., Petcharat, U., Ruangrattanametee, V. and Krisisntu, K. **2000** Control of aflatoxigenic fungi by Thai neem, *Government Pharmaceutical Organization Journal*, 21, 41-49.

[20] Thanaboripat, D., Monkontanawut, N., Suvathi, Y. and Ruangrattanametee, V. **2004** Inhibition of aflatoxin production and growth of *Aspergillus flavus* by citronella oil, *KMITLScience Journal*, 49(1), 1-8.

[21] Thanaboripat, D., Suvathi, Y. and Chantarateptawan, V. **2005** Control of growth and aflatoxin production of *Aspergillus flavus* by medicinal and aromatic plants. In: *Study on Plant Pest and Disease Biological Control and Bio-technology*, pp.52-62. Edited by Yang Qian and Yu Ziniu, Heilongjiang Science and Technology Press, Harbin.

[22] Bankole, S. A. and Joda, A. O. **2004** Effect of lemon grass (*Cymbopogon citrates* Stapf) powder and essential oil on mould deterioration and aflatoxin contamination of melon seeds (*Colocynthis citrullus* L.). *African Journal of Biotechnology*, 3(1), 52-59.

[23] Nunthavutthikul, S. **2000** Studies of microbial inhibition of herbal extracts. Master's thesis, Faculty of Pharmacy Chulalongkorn University, Bangkok.

[24] Lorian, V. **1986** *Antibiotics in Laboratory Medicine*. Lippincott Williams & Wilkins, Philadelphia.

[25] Charoensettasilp, S. **1999** *Experimental Design*. Faculty of Science, King Mongkut's Institute of Technology Ladkrabang., Bangkok.

[26] Brophy, J.J., Thubthimthed, S., Kitirattrakarn, T. and Anantachoke, C. **2002** Volatile leaf oil of *Melaleuca cajuputi*. In *Proceedings of the Forestry Conference*, pp.304-313.

[27] Mahmoud, A.-L.E. **1994** Antifungal action and antiaflatoxigenic properties of some essential oil constituents. *Letters in Applied Microbiology*, 19,110-113.

[28] Ozcan, M. **2005** Effect of spice hydrosols on the growth of *Aspergillus parasiticus* NRRL 2999 strain. *Journal of Medicine Food*, 8(2), 275-278.

[29] Montes-Belmont, R. and Carvajal, M**. 1998** Control of *Aspergillus flavus* in maize with plant essential oils and their components. *Journal of Food Protection*, 61, 616-619.

[30] Zaika, L.L. and Buchanan, R.L. **1987** Review of compounds affecting the biosynthesis or bioregulation of aflatoxins. *Journal of Food Protection*, 50(8), 691-708.

[31] Sinha, K.K., Sinha, A.K. and Prasad, G. **1993** The effect of clove and cinnamon oils on growth of and aflatoxin production by *Aspergillus flavus. Letters in Applied Microbiology*, 16, 114-117.

[32] Conner, D. E. **1993** Naturally occurring compounds, In *Antimicrobial in Foods*, pp.441-468. Edited by Davidson, P. M. and Branen, A. L. Marcel Dekker Inc., New York.

[33] Snyder, O.P. **1997** *Antimicrobial Effects of Spices and Herbs*. Hospitality Institute of Technology and Management, St Paul, Minnisota.

[34] Charles, D. J. and Simon, J.E. **1990** Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *Journal of American Horticultuel Science*, 115, 458-462.

[35] Paakkonen, K., Malmsten, T. And Hyvonen, L. **1990** Drying, packaging and storage effects on quality of basil, marjoram and wild marjoram. *Journal of Food Science*, 55, 1373-1382.

[36] Moretti, M. D. L., Sanna-Passino, G., Demontis, S. and Bazzoni, E. **2002** Essential oil formulations useful as a new tool for insect pest control. *AAPS PharmSciTech*. 3(2), 1-11.