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Antifungal Activity of Clove and Cinnamon Oil and Their Synergistic Against Postharvest Decay Fungi of Grape *in vitro*

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ABSTRACT

The efficacy of essential oil from clove and cinnamon against 6 fungi cuasing postharvest decay of grapes : *Aspergillus niger, Alternaria alternata, Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phomopsis viticola* and *Rhizopus stolonifer* by inverted petriplate method was studied. Antifungal activity of clove oil against all above-mentioned fungi showed Minimal inhibitory concentration (MIC) : 200, 200, 400, 800, 200 and 200 mg/ml, respectively whereas the MIC obtained from cinnamon oil were 50, 100, 200, 200, 100 and 800 mg/ml, respectively. Investigation of synergistic effect of clove and cinnamon oil showed 3 optimum ratios : 3:7, 2:8 and 1:9 and MIC for all fungi obtained from these ratios to inhibit growth of 6 fungi was 400 mg/ml.
Key words: cinnamon oil, clove oil, synergistic action, MIC, FIC

INTRODUCTION

Table grape (*Vitis vinifera* L.) is a non-climacteric fruit with a relatively low rate of physiological activity. Postharvest decay caused by *Rhizopus stolonifer*, *Aspergillus niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides, Lasiodiplodia theobromae* and *Phomopsis viticola* is the major cause of rapid and extensive deterioration of table grapes (Nelson, 1979; Barkai-Golan, 2001; Lichter *et al*., 2002). These pathogens become important at warmer temperatures and they appear commonly sometimes during transportation or marketing after grapes are removed from cold storage. To solve this problem, synthetic fungicides have been used. However, the associations of pesticide usage with the development of fungicide-resistant strains and the public's concern for the human health conditions and the environmental pollution have stimulated the search for new strategies as alternative means for controlling postharvest decay. The advantage of essential oils is their bioactivity in the vapour phase, a characteristic that makes them attractive as possible fumigants for stored product protection. These essential oils are thought to play a role in plant defence mechanisms against phytopathogenic micro-organisms (Mihaliak *et al*., 1991). There are also some reports on essential oils in enhancing storage life of fruits and vegetables by controlling their fungal rotting. Cinnamon oil and clove oil are both natural preservative and flavouring substances that are not harmful when consumed in food products.

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Antimicrobial activity of cinnamon and clove oil have been reported to inhibit the growth of molds, yeasts and bacteria (Matan *et al*., 2006).

The objective of the research was to study the inhibitory effects of cinnamon and clove oils and their synergy on fungi causing postharvest decay of grapes.

MATERIALS AND METHODS

Preparation of essential oils

The essential oils (EO) tested were extracted by the hydrodistillation method using Clevenger's apparatus. They were from *Syzygium aromaticum* (Linn.) Merr.& Perry (dried bud) and *Cinnamonum zeylanicum* (dried bark).The recovered oils were dried over anhydrous sodium sulphate and stored at 4 °C until use.

Isolation and identification

Aspergillus niger, Alternaria alternata, *Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phomopsis viticola* and *Rhizopus stolonifer* were isolated from diseased berries of white Malaga grapes bought from Talaad Thai Market, Bangkok Province, Thailand by tissue transplanting method. Small pieces (5×5 mm), selected from the margins of decay lesions were placed on potato dextrose agar medium (PDA). Pure cultures were obtained by transferring hyphal tips to PDA medium. Isolates were maintained on PDA medium at 4 °C and were identified by macroscopic and microscopic observations by Associate Professor Dr. Somsiri Sangchote (Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Thailand).

Antifungal assays

The antifungal activity of the volatile oil were undertaken using inverted petriplate method (Singh *et al*., 2006). PDA medium plate were prepared using 8.2 cm plastic petri dishes containing 20 ml of PDA. A 5 mm diam. disc of the tested species was cut from the periphery of the active growth culture of PDA medium plates (5-7 days old) and the mycelial surface was placed upside down on the centre of the dish. The 20 µl of undiluted clove and cinnamon oil were pipetted on a small piece (diam. 12 mm.) of Whatman No.1 filter paper and it was kept on the cover lid of petri dish. The tested plates were incubated at room temperature for 7 days, when the mycelium of fungi reached the edges of the control dish, (without essential oils). The antifungal index was calculated as follows : Antifungal index (AI,%) = $(1-D_a/D_b)x100$ where D_a is the diameter of growth zone in the experimental dish (cm) and D_b is the diameter of growth zone in the control dish (cm). (Hsu *et al*., 2007). Each experiment was performed three times and the data were averaged. The results are in Table 1.

Determination of Minimal inhibitory concentration (MIC) and Fractional inhibitory concentration (FIC)

Minimal inhibitory concentration (MIC) for clove and cinnamon oil and their combinations to inhibit fungal growth was determined by inverted petriplate method. The eleven combinations at various ratios were prepared, the following ratios of clove and cinnamon oil were considered 10:0 ; 9:1 ; 8:2 ; 7:3 ; 6:4 ; 5:5 ; 4:6 ; 3:7 ; 2:8 ; 1;9 and 0:10. The concentrations were prepared at 0, 12.5, 50, 100, 200, 400 and 800 mg/ml with dimethyl sulfoxide (DMSO). MIC was defined as the lowest concentration of sample that affected the fungal growth when compared with the growth of control plate. MICs were transformed into fractional inhibitory concentration (FIC) to determine the interaction of clove and cinnamon oil. All ratios were represented in FIC_{index} as suggested by Davidson and Parish (1989):

FIC_A = (MIC of A in presence of B) / (MIC of A) (1)

FIC_B = (MIC of B in presence of A)/(MIC of B) (2)

Fractional inhibitory concentration index (FIC_{index})

was then calculated from FIC values for each antimicrobial as follows:

$FIC_{index} = FIC_A + FIC_B$ (3)

Where A is clove oil, B is cinnamon oil in combinations . The FIC_{index} was interpreted as: a synergistic effect when < 1; additive effect when = 1 and antagonistic effect when >1

Statistical analyses

Statistical analyses of the data were performed with SPSS statistical software.

RESULTS AND DISCUSSION

Antifungal activity of clove and cinnamon oil against *Aspergillus niger, Alternaria alternata Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phomopsis viticola* and *Rhizopus stolonifer* by using inverted petriplate method is reported in Table 1. The results showed that cinnamon and clove oil were non significant to inhibit the growth of *A. alternata, A.niger* and *P.viticola*, while they significantly reduced growth of *C. gloeosporioides, L. theobromae* and *R. stolonifer.* Cinnamon oil at 10 µl was found to be 100% antifungal against all the tested fungi except *R. stolonifer*. Clove oil also showed complete inhibition against all tested fungi. However it had relatively low inhibitory effect on *C. gloeosporioides* and *L. theobromae* whose inhibition indices were 59.12 and 74.07 %, respectively. The efficacy of cinnamon and clove oil as an antifungal agent was reported by Azzouz and Bullerman (1982); Soliman and Badea (2002); Velluti *et al*., (2003); Singh *et al*., (2007); Feng and Zheng (2007); Lopez-Malo *et al*., (2007). The effectiveness against molds of clove and cinnamon oil is affected by their chemical compositions. Eugenol is the main component of clove oil. Antimicrobial activity of this oil can be attributed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with –SH groups in the active sites of target enzymes, resulting in the deactivation of enzymes in fungi (Farag *et al*., 1989; Cowan, 1999; Velluti *et al*., 2003). The main constituent of cinnamon oil is cinnamaldehyde, which is the compound containing an aldehyde group and conjugated double bond outside the ring. This compound possesses much stronger antifungal activity (Wang *et al*., 2005) and it may be a potential lead compound for the development of antifungal drugs through the control β-(1,3)-glucan and chitin synthesis in yeasts and molds (Bang *et al*., 2000)

The MICs of clove and cinnamon oil and their combinations obtained for 6 fungi given in Table 2. The MICs values of cinnamon oil were lower than those obtained from clove oil except *R. stolonifer,* therefore indicating that clove oil gave more effective than cinnamon oil. The interpretation of oil interactions according to Davidson and Parish (1989): defines synergy to occur when $FICin_{dex} < 1.0$ and antagonism when $FIC_{index} > 1.0$, additivity occurs when $FIC_{index} = 1.0$. The results of fractional inhibitory concentration indices of clove and cinnamon oil and their combinations at different ratios against 6 fungi are shown in Table 3. The fractional inhibitory concentration indices ranged between 0.65 and

Table 1 Antifungal investigations of cinnamon and clove oil by using inverted petriplate method.

Essential oil (10µl)	Antifungal index (%)[1]					
	Alternaria alternata	*Aspergillus niger*	*Colletotrichum gloeospo-rioides*	*Lasiodiplodia theobromae*	*Phomopsis viticola*	*Rhizopus stolonifer*
Cinnamon	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	0.00[b]
Clove	100.00[a]	100.00[a]	59.12[b]	74.07[b]	91.00[ab]	100[a]

[1] Antifungal Index are given as mean of three replicates.

Means values within row followed by the same letter are non-significantly different at the 95% confidence level

1.90 for the all fungal tested. Synergistic actions were obtained against *P. viticola* at all ratios, while ratios 4:6, 3:7, 2:8 and 1:9 were synergistic against *A. alternata, A. niger, C. gloeosporiodes, L. theobromae* and *R. stolonifer* except for *A. niger* at ratio 4:6 that showed antagonistic effect. Mostly synergistic actions were obtained with increasing the ratios of cinnamon oil. These findings were quite similar with the results of Matan *et al*., (2006). However, increasing the ratios of clove oil (9:1, 8:2 and 7:3) showed synergistic actions against *R. stolonifer.* This suggested that the two oils in combination were more effective against all fungi than when they were used separately.

Table 2 Minimum inhibitory concentration of clove and cinnamon oil and their combinations.

Ratios		Minimum inhibitory concentration (mg/ml)					
Clove oil	Cinnamon oil	*Aspergillus niger*	*Alternaria alternata*	*Colletotrichum gloeosporiodes*	*Lasiodiplodia theobromae*	*Phomopsis viticola*	*Rhizopus stolonifer*
10	0	200	200	400	800	200	200
9	1	200	200	400	800	100	200
8	2	200	200	400	800	100	200
7	3	200	200	400	800	100	200
6	4	100	100	400	400	100	400
5	5	100	100	400	400	100	400
4	6	100	100	200	200	100	400
3	7	50	100	200	200	100	400
2	8	50	100	200	200	100	400
1	9	50	100	200	200	100	400
0	10	50	100	200	200	100	800

Table 3 Fractional inhibitory concentration index of clove oil and cinnamon oil and their combinations against the tested fungi.

Ratios		Fractional inhibitory concentration index (FIC_{index})					
Clove oil	Cinnamon oil	*Aspergillus niger*	*Alternaria alternata*	*Colletotrichum gloeosporiodes*	*Lasiodiplodia theobromae*	*Phomopsis viticola*	*Rhizopus stolonifer*
10	0	1.00	1.00	1.00	1.00	1.00	1
9	1	1.30	1.10	1.10	1.30	0.55	0.93
8	2	1.60	1.20	1.20	1.60	0.60	0.85
7	3	1.90	1.30	1.30	1.90	0.65	0.78
6	4	1.10	0.70	1.40	1.10	0.70	1.40
5	5	1.25	0.75	1.50	1.25	0.75	1.25
4	6	1.40	0.80	0.80	0.70	0.80	1.10
3	7	0.78	0.85	0.85	0.78	0.85	0.95
2	8	0.85	0.90	0.90	0.85	0.90	0.80
1	9	0.93	0.95	0.95	0.93	0.95	0.65
0	10	1.00	1.00	1.00	1.00	1.00	1.00

The FIC_{index} was interpreted as: a synergistic effect when < 1; additive effect when = 1 and antagonistic effect when >1

CONCLUSIONS

The efficacy of combinations of cinnamon and clove oil showed good potential to inhibit growth of all fungi causing postharvest decay of grapes. To control the growth of these fungi effectively by binary mixture of clove oil and cinnamon oil required higher ratios of cinnamon to clove oil. The optimum ratios of clove and cinnamon oil to control growth of these fungi were 3:7, 2:8 and 1:9 and their MIC was 400 mg/ml.

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LITERATURE CITED

Azzouz, M. A. and L. R. Bullerman. 1982. Comparative antimycotic effects of selected herbs and spices, plant components and commercial antifungal agents. **J. Food Protect.** 45: 1248-1301.

Bang, K.H., D.W.Lee, H.M. Park and Y.H. Rhee. 2000. Inhibition of fungal cell wall synthesizing enzymes by trans-cinnamaldehyde. **Biosci. Biotech. Biochem.** 64: 1061–1063.

Barkai-Golan, R. 2001. **Postharvest Diseases of Fruits and Vegetables Development and Control.** The Netherlands: Elsevier. 418 p.

Cowan, M.M. 1999. Plant Products as Antimicrobial Agents. **Clinical Microbiology Reviews** 12(4): 564–582.

Davidson, P. M. and M. E. Parish. 1989. Methods for testing the efficacy of food antimicrobials. **Food Technol.** 43: 148–155.

Farag, R.S., Z.Y. Daw and S.H. Abo-Raya. 1989. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. **J. Food Sci.** 54: 74–76.

Feng, W. and X. Zheng. 2007. Essential oils to control *Alternaria alternata* in vitro and in vivo. **Food Control** 18: 1126–1130.

Hsu, F.L., H.T. Chang, S.T. Chang. 2007. Evaluation of antifungal properties of octyl gallate and its synergy with cinnamaldehyde. **Bioresour.Technol.** 98: 734–738.

Lichter, A., Y. Zutchy, L.Sonego, O. Dvir, T. Kaplunov, P. Sarig and R. Ben-Arie. 2002. Ethanol controls postharvest decay of table grapes. **Postharvest Biol. Technol.** 24: 301–308.

Lopez-Malo, A., J. Barreto-Valdivieso, E. Palou, F.S. Mart1n. 2007. *Aspergillus flavus* growth response to cinnamon extract and sodium benzoate mixtures. **Food Control** 18: 1358–1362.

Matan, N., H. Rimkeeree, A.J. Mawson , P. Chompreeda, V. Haruthaithanasan and M. Parker. 2006. Antimicrobial activity of cinnamon and clove oils under modifiedatmosphere conditions. **J. Food Microbiol.** 107: 180 – 185.

Mihaliak, C.A., J. Gershenzo and R. Croteau. 1991. Lack of rapidmonoterpene turnover in rooted plants, implications for theories of plant chemical defense. **Oecologia** 87: 373–376.

Nelson, K.E. 1979. **Harvesting and handling California table grapes for market.** University of California. Bulletin no. 1913.

Singh G., M. Sumitra, M.P. de Lampasona and C. Catalan. 2006. Chemical constituents, antifungal and antioxidative potential of

Foeniculum vulgare volatile oil and its acetone extract. **Food Control** 17: 745–752.

____. 2007. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils,oleoresins and their constituents. **Food Chem Toxicol.** 45: 1650–1661.

Soliman, K.M. and R.I. Badea. 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. **Food Chem Toxicol.** 40: 1669–1675.

Velluti, A., V. Sanchis, A.J. Ramos, J. Egido and S. Mar1n. 2003. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. **J. Food Microbiol.** 89: 145– 154.

Wang, S.Y., P.F. Chen and S.T. Chang. 2005. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi **Bioresour. Technol.** 96: 813–818.