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Toxicity of Essential Oils on Toxigenic and Nontoxigenic Fungi

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A large number of naturally occurring compounds, such as essential oils from plants, possess antifungal activity. Reports on the fungicidal and fungistatic activities of essential oils have shown that many of the oils are indeed potent inhibitors of fungal growth. In several studies essential oils have been reported to be effective against toxigenic and nontoxigenic fungi (Maruzzella and Liquori 1958; Maruzzella and Balter 1959; Barnes 1963; Calpouzos 1966; Aulakh and Grover 1968; Bullerman 1974; Bullerman et al. 1977; Moore and Atkins 1977; Sharma et al. 1979; Hitokoto et al. 1980; Sumbali and Mehrotra 1980; Buchanan and Shepherd 1981; Patel et al. 1983; Tiwari et al. 1983; Conner and Beuchat 1984).

The present investigation was designed to evaluate the effectiveness of forty essential oils as fungicidal agents on the mycelial growth of *Rhizopus*, *Mucor* and *Aspergillus* species.

MATERIALS AND METHODS

Twenty fungi, which represented the Plectomycetes and Zygomycetes, were tested for their sensitivity to essential oils. The fungal species, *Rhizopus*, *Mucor* and *Aspergillus*, were obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.) and the Department of National Health and Welfare (Ottawa, Canada). Stock cultures were maintained on slants of potato dextrose agar. Routine subcultures were made every four weeks.

Food-grade quality essential oils of spices were provided by Fritzsche, Dodge and Olcott (New York, New York). Included in the study were essential oils of almond, amyris, anise, basil, bay, bergamot, caraway, castor, cinnamon (bark), cinnamon (leaf), clove (bud), coconut, copaiba, coriander, corn, cottonseed, dill, gennel, garlic, geranium, grapefruit, laurel, lemon, lime, marjoram, nutmeg, olive, onion, orange, pepper (black), peppermint, pimenta (berries), pimenta (leaf), petitgrain, rosemary, sage, sesame, tangerine, thymine and vetiver.

The poisoned food technique of Grover and Moore (1962) was used to test the effectiveness of the essential oils against the fungal species. Each essential oil was diluted in 95% ethanol to give solutions of 0.1, 0.05 and 0.01% (v/v). The appropriate volume of each essential oil was added to 10 ml of sterilized potato dextrose agar medium and swirled properly. A mycelial disc of 6 mm in diameter, cut from the periphery of a 5-day old culture, was aseptically inoculated upside down in the center of the petri plate. For the controls, 0.1 ml of sterilized distilled water and 0.1 ml of 95% alcohol respectively, were mixed with the medium in place of essential oil. The plates were incubated at 27 C and observations recorded after two and five days respectively of incubation.

Radial linear growth was determined by measuring colony diameters at two positions on the fungal colony. Growth inhibition was calculated as: 100 -(radial growth on essential oil-supplemented medium X 100)/(radial growth of the unsupplemented correspounding medium (Solel and Pinkas 1984). Three replicate plates per treatment were used and the experiments were repeated two times.

RESULTS AND DISCUSSION

Forty essential oils were screened for antifungal activity on the mycelial growth of twenty species of fungi, many of which are known as spoilage fungi. Of the forty essential oils, seven were found to be most effective against the test fungi. As shown in Tables 1 and 2, the essential oils of bay, cinnamon (bark, cinnamon (leaf), clove, pimenta (berries), pimenta (leaf) and thyme exhibited complete mycelial growth at 1,000 and 500 ppm respectively. However, clove oil, the most effective of the seven oils also exhibited complete mycelial inhibition at 100 ppm on all of the test fungi.

The results revealed in this investigation suggest that the susceptibility of the spoilage filamentous toxigenic and non-toxigenic fungi to the seven essential oils may have potential application for the control of these fungi. This control, while limited and useful on a small scale, could possible be employed against the growth of non-toxigenic and toxigenic fungi in storage. The essential oils may also prove to be useful sources of fungitoxic

Table 1. Inhibition of Mycelial Growth of *Rhizopus* Species by Essential Oils

Essential Oils	PPM	% Inhibition of Mycelial Growth									
		R. arrhizus	*R. chinensis*	*R. circinans*	*R. japonicus*	*R. kazanensis*	*R. oryzae*	*R. pymacus*	*R. stolonifer*	*R. tritici*	*R.* 66-81-2
Bay	1000	100	100	100	100	40	100	100	100	100	100
	500	100	100	100	100	00	100	100	100	100	37
	100	00	00	81	00	00	00	00	00	37	00
Cinnamon	1000	100	100	100	100	100	100	100	100	100	100
(Bark)	500	100	100	100	00	100	00	100	100	00	00
	100	00	00	00	00	00	00	20	00	00	00
Cinnamon	1000	100	100	100	100	100	100	100	100	100	100
(Leaf)	500	100	100	100	100	100	100	100	100	100	100
	100	00	100	00	00	00	00	00	00	00	00
Clove	1000	100	100	100	100	100	100	100	100	100	100
	500	100	100	100	100	100	100	100	100	100	100
	100	100	100	100	100	100	100	100	100	100	100
Pimenta	1000	100	100	100	100	100	100	100	100	100	100
(Berries)	500	100	100	100	100	100	100	100	100	100	100
	100	00	00	00	00	00	00	00	00	00	00
Pimenta	1000	100	100	100	100	100	100	100	100	100	100
(Leaf)	500	100	100	100	100	100	100	100	100	100	100
	100	00	25	00	100	00	00	100	68	00	00
Thyme	1000	100	100	100	100	100	100	100	100	100	100
	500	100	100	100	100	00	00	100	100	100	100
	100	00	00	00	00	00	00	61	00	00	00

Table 2. Inhibition of Mycelial Growth of _Mucor_ and _Aspergillus_ Species by Essential Oils

Essential Oils	PPM	% Inhibition of Mycelial Growth									
		M. hiemalis	_M. mucedo_	_M. racemosus_ f. _racemosus_	_A. flavus_ 15546[a]	_A. flavus_ 15548[a]	_A. flavus_ 26539[a]	_A. flavus_ 28539[a]	_A. flavus_ 36182[a]	_A. parasiticus_ 26862[a]	_A. parasiticus_ 28285[a]
Bay	1000	100	100	100	d	d	d	d	d	d	d
	500	100	100	100	c	d	d	d	d	d	d
	100	00	00	00	c	c	c	c	c	c	c
Cinnamon	1000	100	100	100	d	d	d	d	d	d	d
(Bark)	500	100	00	00	b	b	b	b	b	b	b
	100	83	00	00	c	c	c	c	c	c	c
Cinnamon	1000	100	100	100	d	d	d	d	d	d	d
(Leaf)	500	100	100	100	b	d	d	d	d	d	d
	100	00	00	50	c	c	c	c	c	c	c
Clove	1000	100	100	100	d	d	d	d	d	d	d
	500	100	100	100	d	d	d	d	d	d	d
	100	100	100	100	d	d	d	d	d	d	d
Pimenta	1000	100	100	100	d	d	d	d	d	d	d
(Berries)	500	100	80	00	c	b	c	b	b	b	d
	100	00	00	00	c	c	c	c	c	c	c
Pimenta	1000	100	100	100	d	d	d	d	d	d	d
(Leaf)	500	100	100	100	d	d	d	d	d	d	d
	100	83	60	100	b	b	b	b	b	b	b
Thyme	1000	100	100	100	d	d	d	d	d	d	d
	500	100	100	100	d	d	d	d	d	d	d
	100	00	100	00	c	b	c	c	c	c	b

[a] Fungus which does not form definite colony

[b] Minimum mycelial growth

[c] Maximum mycelial growth

[d] Complete mycelial growth inhibition

substances that are rather harmless compared to synthetic fungicides which often impose undesirable side effects to humans.

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