

Evaluation of Some Essential Oils for Their Toxicity against Fungi Causing Deterioration of Stored Food Commodities

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During screening of essential oils for their antifungal activities against *Aspergillus flavus*, the essential oil of *Cymbopogon citratus* was found to exhibit fungitoxicity. The MIC of the oil was found to be 1,000 ppm, at which it showed its fungistatic nature, wide fungitoxic spectrum, nonphytotoxic nature, and superiority over synthetic fungicides, i.e., Agrosan G. N., Thiride, Ceresan, Dithane M-45, Agrozim, Bavistin, Emison, Thiovit, wettable sulfur, and copper oxychloride. The fungitoxic potency of the oil remained unaltered for 7 months of storage and upon introduction of high doses of inoculum of the test fungus. It was thermostable in nature with treatment at 5 to 100°C. These findings thus indicate the possibility of exploitation of the essential oil of *C. citratus* as an effective inhibitor of storage fungi.

Fungi are significant destroyers of foodstuffs during storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Storage fungi are commonly controlled by synthetic chemicals; however, most of the fungicides of this group create several side effects in the forms of carcinogenicity, teratogenicity, and residual toxicity (1, 10). Therefore, some alternative biodegradable chemical control measures should be discovered to replace synthetic pesticides for pest management without creating any pesticidal pollution. Recently, in different parts of the world, attention has been paid to exploiting higher-order plant products as novel chemotherapeutants in plant protection. Because of nonphytotoxicity, systemicity (11), easy biodegradability (2), and the stimulatory nature of host metabolism (9, 16, 26), plant products possess the potential to be of value in pest management (4, 5, 7, 8, 12, 14, 17, 22, 24). Therefore, in the present work, it has been thought desirable to discover the antifungal potencies of some higher-order plant products against *Aspergillus flavus*, a dominant, mycotoxin-producing storage fungus. Since the volatile chemicals may have better applicability as fumigants for control of storage diseases, in the present project some essential oils have been tested to examine their usefulness as herbal fumigants.

MATERIALS AND METHODS

Collection of oils. Fresh parts of 14 species of higher-order plants growing luxuriantly in the Ayurvedic garden of the Banaras Hindu University campus were collected in the months of July and August, and their volatile fractions (essential oils) were isolated through hydrodistillation with Clevenger's apparatus (15). The oils were stored separately at $6 \pm 2^\circ\text{C}$ after dehydration.

Toxicity studies. The toxicities of the essential oils against the test fungus were tested at 3,000 ppm by the poisonous-medium technique described by Grover and Moore (13) with potato dextrose agar medium. In treatment plates, the requisite amount of oil was added to potato dextrose agar medium to produce a concentration of 3,000 ppm. Control plates contained equivalent amounts of distilled water. Toxicities were calculated according to the method described by Pandey

et al. (21) and were recorded in terms of percentages of mycelial inhibition. The data presented are based on the mean values of five replicates.

MICs. The percent recoveries of the cymbopogon oil as well as the MICs were determined for each month of the year. For the determination of MICs, the poisonous-medium technique was followed; according to this technique, different concentrations of the oil, i.e., 500, 1,000, and 1,500 ppm, were prepared by supplementing the oil's requisite amount in petri dishes containing potato dextrose agar (PDA) medium. A mycelial disc (5-mm diameter) taken from the 7-day-old culture of the test fungus was inoculated to each petri dish. Plates containing nonpoisoned medium served as control. Fungal colony diameters, in control as well as in treatment sets, were recorded after incubation for 7 days at $25 \pm 2^\circ\text{C}$.

Fungal toxicity. To detect the mycocidal-mycostatic nature of cymbopogon oil, the technique prescribed by Thompson (23) was followed, in which oil-inhibited fungal discs were washed with distilled water and reinoculated onto the fresh medium and the revival of fungal growth was recorded. The mycotoxic spectrum of the oil was evaluated against 47 fungi (obtained from the Indian Agricultural Research Institute, New Delhi, India) at hypotoxic, toxic, and hypertoxic concentrations of the oil (500, 1,000 and 1,500 ppm, respectively) by the usual poisonous-medium technique. In each case, PDA medium was used, except with *Trichophyton mentagrophytes* and *Microsporum gypseum*, for which Sabouraud dextrose agar medium was used. The shelf life (effect of storage on fungitoxicity) at room temperature ($25 \pm 2^\circ\text{C}$), thermostability, effect of inoculum density of the test fungus on the potency of the cymbopogon oil, and comparison of its efficacy with those of some prevalent synthetic fungicides which were obtained from local markets were also determined by the usual poisonous-medium technique described by Grover and Moore (13). For determination of shelf life, the oil stored in an airtight specimen tube at room temperature ($25 \pm 2^\circ\text{C}$) was subjected to fungitoxic testing at its MIC at regular intervals of 1 month. To study the effect of temperature, five lots of oil, each containing 2 ml of the oil, were kept separately in airtight glass vials and were treated at different temperatures, i.e., 5, 10, 40, 80, and 100°C , for 2 h. The oil of each tube was tested for its fungitoxicity at its MIC. To study the effect of increased inoculum density, the oil at its MIC was tested for fungitoxicity

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TABLE 1. Fungitoxicity of essential oils of higher plant parts at 3,000 ppm against *A. flavus*

Source of essential oil tested ^a	Common name (English)	% Inhibition of fungal growth (mean \pm SD)
<i>Ageratum conyzoides</i> (Lf)	Appa grass	90 \pm 2.8
<i>Alpinia carinata</i> (Lf)	Galangal	80 \pm 2.1
<i>Boswellia serrata</i> (Br)	Indian frankincense	50 \pm 1.7
<i>Citrus aurantifolia</i> (Lf)	Lime	80 \pm 3.9
<i>Citrus reticulata</i> (Pl)	Mandarin orange	40 \pm 1.9
<i>Citrus sinensis</i> (Pl)	Sweet orange	60 \pm 1.3
<i>Curcuma longa</i> (Rh)	Turmeric	72 \pm 0.6
<i>Cymbopogon citratus</i> (Lf)	Lemongrass	100
<i>Eupatorium cannabinum</i> (Lf)	Hemp agrimony	42 \pm 1.4
<i>Hyptis suaveolens</i> (Lf)		85 \pm 2.0
<i>Juniperus communis</i> (Lf)	Common juniper	45 \pm 1.1
<i>Mentha viridis</i> (Lf)	Spearmint, wildmint, or brownmint	85 \pm 3.4
<i>Pinus</i> sp. (Lf)	Pine	84 \pm 1.7
<i>Vetiveria zizanioides</i> (Rt)	Vetiver or Khuskhus	56 \pm 0.6

^a Lf, leaf; Br, bark; Pl, peel; Rh, rhizome; Rt, root.

against an increasing number of fungal discs (up to 64 discs) of *A. flavus*.

All of the experiments were done with three replicates.

Phytotoxicity. The phytotoxicity of the oil with respect to seed germination and seedling growth of wheat variety UP 262 and rice variety Sita was assayed by the technique followed by Dikshit et al. (6). Seeds soaked in the toxic concentration of oil (prepared in distilled water) for 30 and 60 min in the case of wheat and 3 h in the case of rice were placed on double-layered moistened Whatman no. 1 filter paper in sterilized petri plates. Seeds soaked only in distilled water served as the control. All of the sets were incubated at ambient temperature ($28 \pm 5^\circ\text{C}$), and percent seed germinations as well as seedling growth were recorded at different intervals. The data presented are based on the mean values of five replicates each with 100 seeds.

RESULTS

During screening of essential oils isolated from different parts of 14 species of higher-order plants at 3,000 ppm, only the essential oil of *Cymbopogon citratus* (family Poaceae) possessed absolute toxicity against *A. flavus*. The percent inhibitions exhibited by the different plant species varied

TABLE 2. Recoveries and MICs of cymbopogon oil isolated in different months of the year against *A. flavus*

Month	% Recovery of oil (mean \pm SD)	% Inhibition of fungal growth at the following concns (mean \pm SD):		
		500 ppm	1,000 ppm	1,500 ppm
January	0.29 \pm 0.014	38 \pm 0.0	80 \pm 3.7	100
February	0.40 \pm 0.016	35 \pm 0.8	73 \pm 0.8	95 \pm 1.4
March	0.46 \pm 0.008	40 \pm 4.3	80 \pm 4.3	98 \pm 2.2
April	0.50 \pm 0.021	50 \pm 2.2	80 \pm 0.0	98 \pm 0.0
May	0.58 \pm 0.0	50 \pm 0.0	100	100
June	0.64 \pm 0.016	52 \pm 2.8	100	100
July	0.60 \pm 0.014	52 \pm 1.6	100	100
August	0.58 \pm 0.014	52 \pm 1.3	100	100
September	0.45 \pm 0.0	50 \pm 0.0	100	100
October	0.38 \pm 0.033	50 \pm 0.8	100	100
November	0.35 \pm 0.008	50 \pm 4.3	100	100
December	0.30 \pm 0.131	48 \pm 3.4	92 \pm 0.8	100

TABLE 3. Range of toxicities of cymbopogon oil at hypotoxic, toxic, and hypertoxic concentrations

Fungus tested	% Fungal inhibition at the following concns (mean \pm SD):		
	500 ppm	1,000 ppm	1,500 ppm
<i>Absidia ramosa</i>	100	100	100
<i>Alternaria alternata</i>	85 \pm 3.7	100	100
<i>Alternaria solani</i>	100	100	100
<i>Alternaria tenuissima</i>	100	100	100
<i>Arthrinium</i> sp.	100	100	100
<i>Aspergillus candidus</i>	98 \pm 1.6	100	100
<i>Aspergillus fumigatus</i>	85 \pm 4.2	96 \pm 2.2	100
<i>Aspergillus koningi</i>	64 \pm 0.0	100	100
<i>Aspergillus luchuensis</i>	63 \pm 2.5	100	100
<i>Aspergillus nidulans</i>	90 \pm 4.1	100	100
<i>Aspergillus niger</i>	100	100	100
<i>Aspergillus okazakii</i>	70 \pm 2.2	95 \pm 2.2	100
<i>Aspergillus parasciticus</i>	100	100	100
<i>Aspergillus sulphureus</i>	65 \pm 0.8	90 \pm 0.8	100
<i>Aspergillus terreus</i>	80 \pm 5.7	100	100
<i>Candida</i> sp.	100	100	100
<i>Cephalosporium humicola</i>	100	100	100
<i>Chaetomium globosum</i>	100	100	100
<i>Cladosporium cladosporioides</i>	100	100	100
<i>Cladosporium herbarum</i>	100	100	100
<i>Cladosporium oxysporum</i>	100	100	100
<i>Colletotrichum falcatum</i>	100	100	100
<i>Curvularia lunata</i>	100	100	100
<i>Drechslera australiensis</i>	100	100	100
<i>Fusarium moniliforme</i>	90 \pm 0.0	100	100
<i>Fusarium nivale</i>	100	100	100
<i>Fusarium oxysporum</i>	100	100	100
<i>Fusarium oxysporum-circinalis</i>	100	100	100
<i>Fusarium semitectum</i>	100	100	100
<i>Fusarium solani</i>	100	100	100
<i>Fusarium udum</i>	58 \pm 2.8	100	100
<i>Helminthosporium oryzae</i>	100	100	100
<i>Microphomina phaseoli</i>	100	100	100
<i>Microsporium gypseum</i>	100	100	100
<i>Mucor</i> sp.	100	100	100
<i>Nigrospora cephalinis</i>	100	100	100
<i>Penicillium citrinum</i>	100	100	100
<i>Penicillium regulosum</i>	100	100	100
<i>Pestalotia</i> sp.	100	100	100
<i>Pythium aphanidermatum</i>	100	100	100
<i>Pythium debaryanum</i>	100	100	100
<i>Rhizoctonia solani</i>	100	100	100
<i>Rhizopus nigricans</i>	50 \pm 4.1	100	100
<i>Trichoderma viride</i>	45 \pm 2.2	100	100
<i>Trichophyton mentagrophytes</i>	100	100	100
<i>Trichothecium</i> sp.	100	100	100
<i>Verticillium</i> sp.	100	100	100

considerably, ranging from 40% (*Citrus reticulata*) to 100% (*C. citratus*) (Table 1). Therefore, cymbopogon oil was selected for further studies. Maximum recovery of cymbopogon oil was found in June (0.64%), and minimum recovery was recorded in January (0.29%). During the fungitoxic assay, the cymbopogon oil isolated in the months of May to November exhibited the maximum antifungal potency even at 1,000 ppm, while the oils isolated during December and January were 100% inhibitory only at 1,500 ppm. The oils isolated in February to April could not completely inhibit the growth of test fungus even at 1,500 ppm; thus, the fungitoxic potencies of the oil varied with different months of isolation during the year (Table 2). The MIC of cymbopogon oil for complete inhibition of *A. flavus*

TABLE 4. MICs and comparative efficacy of cymbopogon oil and of some synthetic fungicides

Fungicide or oil	% Inhibition of growth of <i>A. flavus</i> at the following concns (mean \pm SD):								
	500 ppm	1,000 ppm	2,000 ppm	3,000 ppm	4,000 ppm	5,000 ppm	6,000 ppm	7,000 ppm	8,000 ppm
Agrosan G.N.	28 \pm 1.6	50 \pm 4.3	80 \pm 2.2	84 \pm 2.8	100 ^a	100	100	100	100
Agrozim	13 \pm 1.4	22 \pm 1.4	50 \pm 1.6	61 \pm 2.9	83 \pm 1.6	98 \pm 1.6	100 ^a	100	100
Bavistin	11 \pm 0.8	20 \pm 1.6	37 \pm 1.4	76 \pm 2.4	85 \pm 2.4	94 \pm 2.8	100 ^a	100	100
Ceresan	4 \pm 0.8	7 \pm 1.9	31 \pm 2.2	64 \pm 1.6	80 \pm 2.2	100 ^a	100	100	100
Copper oxychloride	0 \pm 0	6 \pm 2.2	16 \pm 1.4	25 \pm 2.4	46 \pm 3.3	57 \pm 1.6	66 \pm 2.9	90 \pm 1.6	100 ^a
Dithane M-45	10 \pm 1.4	27 \pm 2.9	50 \pm 0.8	62 \pm 2.2	81 \pm 2.4	100 ^a	100	100	100
Emison	8 \pm 0.8	18 \pm 2.4	29 \pm 1.6	68 \pm 2.2	80 \pm 0.8	98 \pm 1.6	100 ^a	100	100
Thiovit	10 \pm 1.9	29 \pm 2.9	51 \pm 0.8	67 \pm 1.6	81 \pm 2.4	94 \pm 1.4	100 ^a	100	100
Wettable sulfur	0 \pm 0	12 \pm 1.9	21 \pm 2.9	29 \pm 2.9	58 \pm 4.9	75 \pm 3.6	94 \pm 3.3	100 ^a	100
Cymbopogon oil	52 \pm 0.8	100 ^a	100	100	100	100	100	100	100

^a MIC.

was found to be 1,000 ppm. The oil at its lower concentration (500 ppm) showed a maximum of 52% inhibition. At the MIC (1,000 ppm), the oil exhibited its fungistatic nature but turned fungicidal at 2,000 ppm. The cymbopogon oil exhibited a broad spectrum of fungitoxicity by inhibiting completely growth of 35, 45, and 47 fungal species at 500, 1,000, and 1,500 ppm, respectively (Table 3), and its fungitoxic potency remained unaltered for 210 days of storage, after which it started to decline. Moreover, cymbopogon oil exhibited the thermostable nature of its fungitoxicity at concentrations of 1,000 and 1,500 ppm, since it exhibited absolute inhibition of fungal growth following all temperature treatments from 5 to 100°C. At a concentration of 1,000 ppm, the oil inhibited 64 fungal discs of *A. flavus* (approximate number of spores per dish 10^{9.18}); thus, the increased density of the inoculum of the test fungus could not alter the potency of cymbopogon oil. The oil was found to be more efficacious than the 10 synthetic fungicides tested. It was four times superior to Agrosan G.N. (organomercurial dust) and Thiride (tetramethylthiuram disulfide); five times superior to Ceresan (mercury) and Dithane M-45 (mancozeb); six times superior to Agrozim (carbendozim), Bavistin (carbendozim), Emison (mercury), and Thiovit; seven times superior to wettable sulfur (micronized sulfur); and eight times superior to copper oxychloride (Blitox-50) (Table 4). Moreover, the oil was nonphytotoxic in nature, since it did not exhibit any adverse effects on germination and seedling growth of wheat and rice (Tables 5 and 6).

DISCUSSION

Thus, these findings indicate that cymbopogon oil is an effective postharvest fungitoxicant of higher-order plant origin potentially suitable for protection of foodstuffs from storage fungi. Because of its broad antifungal spectrum, superiority over synthetic chemicals, availability, and renewable nature,

the cymbopogon oil appears to be economical in its application. The retention of its fungitoxic potency for a long duration at room temperature and at temperatures ranging from 5 to 100°C will facilitate future isolation of the active components of the oil.

Monthly variation in the fungitoxic potencies of cymbopogon oil was observed in the present study, and the oil from May to November was found to be the most potent. This may be because of the presence of larger quantities of active substances in *Cymbopogon* leaves in these months; therefore, the oil of this period should be trapped for use. Such information regarding variation of fungitoxic potency with the seasons and with the growth stages of plants should be recorded for any higher-order plants showing biological activity. Although several essential oils have been tested against storage fungi, the MIC of cymbopogon oil (1,000 ppm) was lower than those of most of the other oils, i.e., *Ocimum basilicum* (7), *Callistemon lanceolatus* (20), *Alpinia galanga* (25), *Ocimum canum* (3), *Cinnamomum camphora* (18), *Amomum subulatum* (17), and *Citrus aurantifolia* (27). *C. citratus* is widely cultivated in India and is thus an available and renewable resource. When tested for its animal toxicity by feeding it to albino rats, cymbopogon oil exhibited no animal toxicity (19). Thus, the use of the oil as a toxicant appears to be an economical measure for the protection of food commodities from storage fungi.

Besides their application in different medicines for human health, higher-order plant products may be used in plant protection too, and the volatile plant products (essential oils) may be exploited as herbal fumigants in the protection of stored foodstuffs from storage pests.

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TABLE 5. Effect of cymbopogon oil on germination of seeds of wheat and rice

Germination period (h)	% Germination of wheat (mean \pm SD)				% Germination of rice (mean \pm SD) with 3-h treatment ($t = 0.014$) ^a	
	1/2-h treatment ($t = 0.447$) ^a		1-h treatment ($t = 0.779$) ^a		Control	Treatment
	Control	Treatment	Control	Treatment		
24	90 \pm 2.3	95 \pm 2.6	95 \pm 0.6	50 \pm 3.5	1 \pm 0.6	0 \pm 0.0
48	100	100	98 \pm 0.6	100	96 \pm 2.5	96 \pm 1.8
72	100	100	98 \pm 0.6	100	100	99 \pm 0.8
96	100	100	98 \pm 0.6	100	100	100

^a Tabulated value of t at 5% level of significance at 6 df is 2.447. Differences are not significant.

TABLE 6. Effect of cymbopogon oil on seedling growth of wheat and rice^a

Age of seedlings (h)	Mean length of wheat seedlings \pm SD (cm) in the:						Mean length of rice seedlings \pm SD with 3-h treatment in the:																
	1/2-h treatment			1-h treatment			C			T													
	C	T	P ($t = 0.238$) ^b	C	T	R ($t = 0.096$) ^b	C	T	P ($t = 0.57$) ^b	C	T	R ($t = 0.035$) ^b	C	T	P ($t = 0.039$) ^b								
48	1.1 \pm 0.063	1.2 \pm 0.126	0.6 \pm 0.0	0.6 \pm 0.11	1.5 \pm 0.167	1.3 \pm 0.11	1.0 \pm 0.179	0.6 \pm 0.063	0.8 \pm 0.141	0.7 \pm 0.11	0.4 \pm 0.063	0.3 \pm 0.089	2.2 \pm 0.19	2.5 \pm 0.167	1.7 \pm 0.212	1.8 \pm 0.11	1.9 \pm 0.19	2.1 \pm 0.11	1.5 \pm 0.179	2.2 \pm 0.167	2.1 \pm 0.141	0.9 \pm 0.089	1.0 \pm 0.06
72	2.2 \pm 0.19	2.5 \pm 0.167	2.3 \pm 0.19	2.4 \pm 0.19	2.3 \pm 0.179	2.4 \pm 0.11	2.0 \pm 0.141	1.9 \pm 0.141	3.5 \pm 0.26	3.6 \pm 0.141	2.8 \pm 0.219	2.7 \pm 0.11	2.8 \pm 0.141	2.8 \pm 0.219	2.7 \pm 0.11	2.8 \pm 0.219	2.7 \pm 0.11	2.8 \pm 0.219	2.7 \pm 0.11	2.8 \pm 0.219	2.7 \pm 0.11	2.8 \pm 0.219	2.7 \pm 0.11

^a R, recticle length; P, plumule length; C, control set; T, treatment set.^b Tabulated value of t at 5% level of significance at 4 df is 2.776. Differences are not significant.

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