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RESEARCH ARTICLE

PLANT VOLATILE OILS AS GROWTH INHIBITORS FOR *ASPERGILLUS PARASITICUS*

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ABSTRACT

Twelve different essential oils (camphor, chaulmoogra, cinnamon, eucalyptus, juniper, khus, lavender, lemon, motia rosha, orange, peppermint and rosemary) were tested against the growth of *Aspergillus parasiticus*. This strain produced aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). After screening the antifungal activity, Minimum inhibitory concentrations (MIC) of essential oils were evaluated by food poison technique. An array of intensities of activity against the growth of *A. parasiticus* was determined in time dependent manner. The toxic strain was sensitive to the majority of the oils tested. Among the essential oils tested motia rosha was excellent antifungal at very low concentration of 0.05%. Eight oils like camphor, cinnamon, eucalyptus, khus, lavender, lemon, peppermint and rosemary were growth inhibitory at various incubation periods but the three oils chaulmoogra, juniper and orange were weak inhibitory after 48 hr incubation. The results suggest the power of volatile aroma compounds to use the preparation of these oils as a disinfectant against fungi.

INTRODUCTION

The genus *Aspergillus* is important group of organisms that work both for and against the interest of humanity. Most of the species of *Aspergillus* are saprophytic growing on decaying vegetables, butter, bread, rice, jams, leather, cloths, fabrics etc. They invade virtually every possible habitat and type of substratum. Various *Aspergillus spp*. have the ability to secrete different types of products like enzymes, primary and secondary metabolites (organic acids, aflatoxins). Mycotoxins are non volatile, secondary metabolites of fungi produced on crops and their products. The most well documented mycotoxins are aflatoxins, trichothecenes and ochratoxins (Kilburn, 2004). Aflatoxin B1 is the most thoroughly studied mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Dorner, 2004). As their health hazards aware the current work is marching towards the control of aflatoxins in crop and foodstuffs. Human health effects attributed to inhalation of mycotoxins include acute or chronic liver and central nervous system damage, endocrine effects and cancer (Hawskworth, 1991). Ingestion of mycotoxins in food stuffs can lead to gastrointestinal complaints such as nausea, vomiting and diarrhea. Ingestion of mold contaminated grain has been associated with cancers in humans. In agricultural settings, mycotoxicoses in both farm animals and humans can result from oral, dermal, or inhalant exposure of mycotoxin contaminated grain or dust (Tuomainen *et al.,* 2003). Several products are known to control toxigenic fungi like chemicals, spices and oils derived from plants with antitoxigenic potential. A large number of synthetic fungicides are being used to control the fungal growth on agricultural crops, food and feed commodities. Such chemicals can be persistent or pollutive in nature and may pose potentially serious health risks and toxicity to non target organisms. An alternative to these synthetic fungicides, that could be safe and natural, is plant essential oils to control the growth of pathogenic fungi. Plant essential oils are usually fragrant volatile compounds named after the aromatic properties of plant material from which they are extracted by distillation (Farnsworth et al. 1985). These oils are accumulated in various plant vegetative parts such as leaves (mint, lemon grass, ginger grass, eucalyptus), barks (cinnamon), wood (sandalwood, camphor), seeds (nutmeg), flowers (clove, geranium, lavender, orange, rose, ylang jasmine) and peel (lime, orange) (Deans & Ritchie 1987). In this study twelve different essential oils were tested against the growth of seed borne aflatoxigenic isolate of *Aspergillus parasiticus*. After screening the antifungal activity, Minimum inhibitory concentrations (MIC) of essential oils were evaluated.

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MATERIALS AND METHODS

Culture

Aspergillus parasiticus was isolated from the contaminated cereal grains (rice) and maintained by regular transfers on Czapek's Dox agar (CDA) at 28˚C. Czapek's Dox agar was purchased from Hi - Media Laboratories Pvt. Ltd M.S. India.

Table 1. Effect of plant essential oils on *Aspergillus parasiticus* after 24 hr incubation

Plant essential oil	percentage of oil concentration										
	Control	0.01	0.03	0.05	0.07	0.09	0.1	0.3	0.5	0.7	0.9
Camphor oil	1.4 (0)	1.2 (14.28)	1.0 (28.57)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Chaulmogra oil	1.4 (0)	1.35 (3.5)	1.35 (3.5)	1.3 (7.1)	1.2 (14.28)	1.2 (14.28)	1.2 (14.28)	1.0 (28.57)	0.9 (35.71)	0.0 (100)	0.0 (100)
Cinnamon oil	1.4 (0)	1.1 (21.42)	1.0 (28.57)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Eucalyptus oil	1.4 (0)	1.2 (14.28)	1.0 (28.57)	0.9 (35.71)	0.9 (35.71)	0.8 (42.85)	0.8 (42.85)	0.65 (53.57)	0.0 (100)	0.0 (100)	0.0 (100)
Juniper oil	1.4 (0)	1.2 (14.28)	1.1 (21.42)	1.1 (21.42)	1.1 (21.42)	1.0 (28.57)	0.9 (35.71)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Khus oil	1.4 (0)	1.0 (28.57)	1.0 (28.57)	0.9 (35.71)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Lavender oil	1.4 (0)	1.3 (7.1)	1.2 (14.28)	1.0 (28.57)	0.7 (50)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Lemon oil	1.4 (0)	1.2 (14.28)	1.1 (21.42)	1.0 (28.57)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Motiarosha oil	1.4 (0)	0.9 (35.71)	0.8 (42.85)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Orange oil	1.4 (0)	1.2 (14.28)	1.1 (21.42)	1.0 (28.57)	0.9 (35.71)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Peppermint oil	1.4 (0)	1.2 (14.28)	1.0 (28.57)	1.0 (28.57)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Rosemary oil	1.4 (0)	1.3 (7.14)	1.2 (14.28)	1.0 (28.57)	0.9 (35.71)	0.8 (42.85)	0.7 (50)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)

Radial growth (cm), Values in parenthesis indicate % of growth inhibition

Table 2. Effect of plant essential oils on *Aspergillus parasiticus* after 48 hr incubation

plant essential oil	percentage of oil concentration										
	control	0.01	0.03	0.05	0.07	0.09	0.1	0.3	0.5	0.7	0.9
Camphor oil	2.4 (0)	2.1 (12.5)	1.9 (20.83)	1.5 (37.5)	1.4 (41.66)	1.1 (54.16)	1.0 (58.33)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Chaulmogra oil	2.4 (0)	2.1 (12.5)	2.0 (16.66)	1.7 (29.10)	1.45 (39.58)	1.3 (54.16)	1.25 (47.91)	1.2 (47.91)	1.1(54.16)	1.0(58.33)	0.0 (100)
Cinnamon oil	2.4 (0)	1.85 (22.91)	1.8 (25)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Eucalyptus oil	2.4 (0)	2.2 (8.33)	2.2 (8.33)	2.1 (12.50)	2.15 (10.41)	2.0 (16.66)	1.9 (20.83)	1.6 (33.33)	1.4(41.66)	1 (54.16)	0.0 (100)
Juniper oil	2.4 (0)	1.8 (25)	1.8 (25)	1.7 (29.16)	1.6 (33.33)	1.5 (37.50)	1.5 (37.50)	1.3 (45.83)	1.2 (50)	1.2 (50)	0.0 (100)
Khus oil	2.4 (0)	1.3 (45.83)	1.3 (45.83)	1.2 (50)	1.2 (50)	1.0 (58.33)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Lavender oil	2.4 (0)	2.2 (8.33)	2.0 (16.66)	1.8 (25)	1.4 (41.66)	1.2 (50)	1.0 (58.33)	0.8 (66.66)	0.0 (100)	0.0 (100)	0.0 (100)
Lemon oil	2.4 (0)	2.2 (8.33)	2.1 (12.5)	1.7 (29.10)	1.5 (37.5)	1.5 (37.5)	1.3 (45.80)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Motiarosha oil	2.4 (0)	1.2 (50)	1.0 (58.33)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Orange oil	2.4 (0)	2.0 (16.66)	2.0 (16.66)	1.9 (20.83)	1.8 (25)	1.7 (29.10)	1.4 (41.66)	1.2 (50)	1.0(58.33)	0.0 (100)	0.0 (100)
Peppermint oil	2.4 (0)	1.7 (27.50)	1.6 (33.33)	1.1 (54.16)	0.9 (60.80)	0.7 (70.83)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Rosemary oil	2.4 (0)	2.2 (8.33)	2.0 (16.66)	1.8 (25)	1.6 (33.33)	1.3 (45.83)	1.1 (54.16)	0.8 (66.66)	0.0 (100)	0.0 (100)	0.0 (100)

Radial growth (cm), Values in parenthesis indicate % of growth inhibition

Table 3. Effect of plant essential oils on *Aspergillus parasiticus* after 72 hr incubation

Plant essential oil	percentage of oil concentration										
	control	0.01	0.03	0.05	0.07	0.09	0.1	0.3	0.5	0.7	0.9
Camphor oil	3.6 (0)	3.0 (16.66)	2.9 (19.83)	2.5 (34.5)	1.9 (31.66)	1.7 (52.16)	1.5 (66.63)	1.0 (72.22)	0.0 (100)	0.0 (100)	0.0 (100)
Chaulmogra oil	3.6 (0)	3.2 (11.15)	3.0 (16.66)	3.0 (16.66)	2.9 (19.83)	2.8 (22.22)	2.75 (27.91)	2.7 (37.91)	2.55 (42.16)	2.3 (48.33)	2.0 (49)
Cinnamon oil	3.6 (0)	2.6 (27.91)	2.4 (33.33)	2.2 (38.88)	2.1 (41.66)	2.0 (44.44)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Eucalyptus oil	3.6 (0)	2.9 (18.33)	2.8 (22.33)	2.7 (35.50)	2.7 (35.50)	2.5 (46.66)	2.5 (46.66)	2.46 (53.33)	2.4 (54.66)	2.2 (59.16)	0.0 (100)
Juniper oil	3.6 (0)	2.8 (25)	2.6 (27)	2.5 (30.16)	2.5 (30.16)	2.4 (33.50)	2.3 (36.11)	2.23 (37.83)	2.1 (41.66)	2.1 (41.66)	2.0 (44.44)
Khus oil	3.6 (0)	2.0 (44.44)	1.8 (50.83)	1.7 (52.77)	1.6 (55.55)	1.5 (58.33)	1.4 (61.11)	1.2 (66.66)	1.0 (72.22)	0.0 (100)	0.0 (100)
Lavender oil	3.6 (0)	2.8 (22.33)	2.7 (26.66)	2.4 (35)	2.0 (44.66)	1.8 (50)	1.6 (55.33)	1.3 (63.66)	1.1 (69.44)	1.0 (72.22)	0.0 (100)
Lemon oil	3.6 (0)	2.8 (22.33)	2.5 (30.16)	2.5 (30.16)	2.3 (36.11)	2.23 (37.83)	2.1 (41.66)	1.7 (52.16)	1.2 (66.66)	0.0 (100)	0.0 (100)
Motiarosha oil	3.6 (0)	1.7 (52.16)	1.3 (63.66)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)	0.0 (100)
Orange oil	3.6 (0)	3.2 (11.15)	3.0 (16.66)	2.8 (25)	2.8 (25)	2.6 (27)	2.5 (30.16)	2.4 (33.50)	2.4 (33.50)	2.2 (38.88)	2.0 (44.66)
Peppermint oil	3.6 (0)	2.6 (27.91)	2.4 (35)	2.2 (38.88)	1.6 (55.55)	1.3 (63.66)	1.1 (69.44)	1.0 (72.22)	0.0 (100)	0.0 (100)	0.0 (100)
Rosemary oil	3.6 (0)	3.1 (13.88)	2.9 (18.33)	2.8 (22.33)	2.4 (35)	2.2 (38.88)	2.0 (44.44)	1.6 (55.33)	1.2 (66.66)	1.0 (72.22)	0.0 (100)

Radial growth (cm), Values in parenthesis indicate % of growth inhibition

Aflatoxin extraction and assay

Aflatoxin extraction was carried from *Aspergillus parasiticus* by growing in Yeast extract Sucrose (YES) broth medium (yeast extract – 2 %, sucrose – 15 %, pH – 6) under stationary conditions at 28˚C for 7 days. Triplicate flasks were maintained. The medium was filtered through a siliconised glass wool filter. The biomass was thoroughly washed with distilled water and later blotted with filter paper and weights were recorded. Aflatoxins were extracted from filtrate by solvent extraction procedure. The filtrate was defatted with n-hexane (60˚C) and sodium chloride (80 mg / ml). Aflatoxins were detected on precoated polyester silica gel-G TLC plates of particle size 2 – 25 µ (20 x 20 cm). The TLC plates were activated at 110˚ C for 30 minutes and used for preliminary screening of aflatoxins. The dried chloroform extracts were made up to 1ml with benzene : acetonitrile (98:2) and 10 µl was spotted on TLC plate along with reference aflatoxin standards - AFB_1, AFB_2, AFG_1 and AFG_2. The plates were developed in solvent system (toluene : ethyl acetate: formic acid in 6 : 3 : 1) and observed under long UV (365 nm) to detect aflatoxins. The separated aflatoxins were quantified by fluoro densitometry.

Testing for antifungal activity of plant oils

Plant Oils

1. Camphor (*Cinnamonum camphora*) oil 2. Chaulmoogra (*Hydnocarpus wightiana*) oil 3. Cinnamon (*Cinnamomum verum*) oil 4. Eucalyptus (*Eucalyptus globulus*) oil 5. Juniper (*Juniperus communis*) oil 6. Khus (*Vetiveria ziazaniodes*) oil 7. Lavender (*Lavandula latifolia*) oil 8. Lemon (*Citrus limon*) oil 9. Motia rosha (*Andropogon marthi*) oil 10. Peppermint (*Mentha piperita*) oil 11. Orange (*Citrus sinensis*) oil 12. Rosemary (*Rosmarinus officinalis*) oil. These oils were purchased from local market (Dr Jain's Forest Herbals Pvt Ltd, Mumbai, India-400059). These oils were screened for antifungal activity against *Aspergillus parasiticus* using Poison food technique (Hamburger and Hostettmann 1991). PDA containing various concentrations of oils ranging from 0.1 - 1% was prepared. Tween-80 (0.5%) was added to increase the solubility of oils. Approximately 20 ml agar medium was poured in each plate aseptically. For each concentration, triplicates of plates were prepared. 0.8 cm agar discs of 24 hrs old activated fungal cultures were replaced in the center of plate containing oils with the help of a sterile cork borer and glass rod. All the plates were incubated at 28˚C for 72hrs. Radial growth was measured at 24 hrs interval. The percentage of inhibition was calculated by using the following formula: I = C – T / C X 100, Here C = radial growth in control – disc diameter (cm), T = radial growth in treated – disc diameter (cm), I = percentage of inhibition.

RESULTS AND DISCUSSION

Aspergillus parasiticus produced were aflatoxin B_1 aflatoxin B_2 aflatoxin G_1 and aflatoxin G_2. 82.6 µg / ml of aflatoxin B_1 (AFB_1), 23.2 µg / ml of aflatoxin B_2 (AFB_2), 25.21 µg / ml of aflatoxin G_1 (AFG_1) and 24.5 µg / ml of aflatoxin G_2 were produced in vitro. Exposure through oral or inhalation to such *Aspergillus* strains may be hazardous to human and animal health. Contamination of cereal grains with fungal metabolites by *Aspergillus* strains cause severe loss of agricultural products (Koirala *et al.,* 2005). An estimate has proved that 25 % of the world's cereal crops are damaged with such mycotoxins annually (Galvano *et al.,* 2001).

Among the twelve essential oils screened all of them were inhibitory against *A. parasiticus*. Minimum inhibitory concentrations (MIC) of the twelve oils were determined against the *A. parasiticus*. An array of intensities of activity against the growth of *A. parasiticus* was determined in time dependent manner. The toxic strain was sensitive to the majority of the oils tested. After 24 hr incubation all the oils tested were found to inhibit growth in the concentration range 0.05 – 0.7 % (Table 1). 0.05 – 0.1 % concentration of oils tested were found inhibitory on the growth of test organism at 48 hr incubation (Table 2). 0.05% concentration of oils like cinnamon, motia rosha and camphor inhibited at 24, 48 hr incubation periods. After 72 hr incubation three oils chaulmoogra, juniper and orange were found non inhibitory and the other nine oils were found strongly effective (Table 3). Among the essential oils tested motia rosha was excellent antifungal at very low concentration of 0.05% (Table 1, 2, 3). Result of this study proves potential to use of nine oils as antifungals to inhibit the growth of pathogenic fungi.

Seed borne fungi could be controlled conventionally by use of synthetic chemical fungicides through their application on the grains (Harris *et al.,* 2001, Dukic et al. 2004). Wide spread public awareness on the use of synthetic chemical fungicides show the environmental effects and their restricted use (Lewis *et al.,* 1998). This concern provides opportunity to screen the alternative products that can cut the burden of potentially risky chemical fungicides with natural products like plant extractives (Isman 2000). The essential oils used in this study are used as flavoring (lemon oil), perfumery (rosemary oil) and therapeutic purpose (eucalyptus oil) (Davidson and Parsih 1989). Oils of pepper mint, cinnamon, lavender, camphor and orange are used in aromatherapy (Helliwell and Ransom 1981) hence these oils are best alternatives to control the seed borne fungi. The oils are volatile with pleasant odor that can be used singly or in combinations as broad spectrum antifungals.

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