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Efficacy of Six Plant Essential Oils against Phytopathogenic Fungi *Aspergillus Niger*

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Abstract—*About 85% of plant diseases are caused by fungi. Fungi are significant destroyers of foodstuffs and grains during storage, making them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins. A significant portion of the agricultural produce in the country and the world over become unfit for human consumption due to mycotoxin contamination of grains, especially those produced by species of Aspergillus. A. niger is a saprophyte in soil causes black mould of onion, garlic and shallot; stem rot of Dracaena; boll rot of cotton; spoilage of cashew kernels. Chemical fungicides are the most popular method of plant disease control. However, these cause deleterious effects on humans and the environment. Alternative source of fungicides are being searched throughout the world. The present study shows the inhibitory effect of six essential oils (clove, lemongrass, mentha, eucalyptus, orange and turmeric oil) against A. niger. Fungal growth inhibition bioassay was conducted to test the efficacy of essential oils. Growth inhibition studies confirmed that clove oil, lemongrass, eucalyptus and mentha oil were found to be potent against the fungus. However, turmeric and orange oil were not effective against the fungus.*

Keywords *(Phytopathogenic Fungi, Aspergillus niger, essential oil, Growth inhibition)*

1. INTRODUCTION

Aspergillus niger is the most important species of the *Aspergillus* genus and ubiquitous in nature. It is responsible for food spoilage, industrial and agricultural product mold, and mycotoxin pollution (ochratoxin A). It can also cause otomycosis and pulmonary infections in immune-compromised persons [1-2]. *Aspergillus* spp., grows on an extensive range of organic substrates, and cause deterioration of stored food material [3-5]. Studies reported that *A. niger* induce spoilage of mangoes [6], grapes [7], and tomatoes [8]. *A. niger* also damages kolanuts during storage [9]. This fungus causes a disease known as black mold on several vegetables including onions. The presence of this fungus in food and feed affects human and animal health. Thus, control of this fungus is critical to decrease the postharvest loss of several horticultural crops.

Various fungicides including carbendazim hexaconozole, bitertanol, myclobutanil, mancozeb, captan and zineb are used to control *A. niger* [10]. Synthetic fungicides are presently used as primary means for the control of plant disease. However, the alternative control approaches are required due to their hazardous effects, resistance in fungal pathogens, and high production cost of new chemicals. The utilization of plant-derived natural products has been studied by researchers as disease control agents. They have many advantages like less environmental effects and low mammalian toxicity. Essential oils are concentrated, hydrophobic liquid containing volatile aromatic compounds present in different parts of the plants such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots [11]. The major components of essential oils are mono and sesquiterpenes, carbohydrates, phenols, alcohols, ethers, aldehydes and ketones which accounts for the biological activity and fragrance. Essential oils have antifungal, antibacterial, antiviral, insecticidal and antioxidant properties. They have no adverse effects on humans and animals [12-13].

The volatile oil obtained from fresh leaves of *Cymbopogon citratus* (Lemongrass) plant is widely used in cosmetics and perfumes industries [14]. It has both antibacterial and antifungal activities [15-16]. It contains monoterpenes, and citral (65-85%) as the major component and small amounts of geraniol, geranylacetate and monoterpene olefins, such as myrcene [14].

The *Citrus sinensis* peel essential oils (Orange oil) has wide applications in the confectionary, toiletry and perfumery industry. They show antimicrobial properties such as antifungal, antiviral, antibacterial and antiparasite [17-19]. Limonene was found to be the major component (84.2%) [18].

Clove essential oil (CEO), extracted from the dried flower buds of the clove plant *Syzygium aromaticum*, comprises mainly phenylpropanoids, such as eugenol (76.8%), β-caryophyllene (17.4%), and α-humulene (2.1%) [20]. Eugenol (4-allyl-2-

methoxyphenol) is the main bioactive ingredient of CLO, which has strong insecticidal, antioxidant, and antifungal activity [21-23].

Turmeric oil is obtained from the rhizome of *Curcuma longa*. The aromatic taste and smell of *C. longa* is due to the presence of volatile essential oil in the rhizome. The oil contains eucalyptol (11.2%), α-turmerone (11.1%), β-caryophyllene (9.8%), ar-turmerone (7.3%) and β-sesquiphellandrene (7.1%) as major constituents [24].

Eucalyptus oil from the leaves of *Eucalyptus globulus* constitutes more than 80% cineol, and other components such as p-cymene, alpha-pinene, limonene, geraniol and camphene [25]. The oil and its constituents have also been used for their fungicidal [26], herbicidal [27], insecticidal [28] properties.

Mentha oil is generally used as a flavoring agent in pharmaceuticals, mouthwash, chewing gum, toothpaste and cigarettes and also been used in Eastern and Western traditional medicine as an antispasmodic and antiseptic in the cure of cancers, indigestion, nausea, cramps, colds, sore throat, and toothaches [29]. Menthol (53.28 %), the major compound of Mentha oil [30], exhibited antifungal activities against *Pseudomonas solanacerum*, *Aspergillus niger*, *Alternaria alternata* and *Fusarium chlamydosporum*, respectively [31-33].

The objective of this study was to examine the antifungal activity of six plant essential oils from *Cymbopogon citratus* (Lemon grass oil), *Citrus sinensis* (orange oil), *Curcuma longa* (turmeric oil), *Eucalyptus globulus* (eucalyptus oil), *Mentha piperata* (mentha oil) and *Syzygium aromaticum* (clove oil) against phytopathogenic fungi *Aspergillus niger*.

2. MATERIALS AND METHODS

2.1 Fungal Strain

Aspergillus niger was procured from IARI, New Delhi. The fungus was cultured and sub-cultured using potato dextrose agar medium and kept in refrigerator at 4^{o} C for further testing.

2.2 Essential Oils

Plant essential oils from *Cymbopogon citratus* (Lemon grass oil), *Citrus sinensis* (orange oil), *Mentha piperata* (mentha oil), *Curcuma longa* (turmeric oil), *Eucalyptus globulus* (eucalyptus oil) and *Syzygium aromaticum* (clove oil) were procured from local market.

2.3 Fungal Growth Inhibition Test/ Poisoned Food Technique

To determine the effect of essential oils on growth of fungus, different concentrations of essential oils diluted with acetone in 1:1 ratio were added into Potato dextrose agar media at 0.1, 0.25, 0.5, 0.75, 1, 5, 10 % concentration. Treated media (20 ml) was then poured into the petri plate and allowed to solidify. Mycelial plugs (6 mm in diameter) of pure culture of *A. niger* were incubated in the center of each PDA plate (9 cm diameter). All the experimental transfers were performed aseptically in laminar air flow. These plates inoculated with fungus were incubated in the dark at 28 °C and 70% RH for 7-10 days. Mycelial growth was measured every day until control plates were completely colonized with mycelium. Plates with only media and no oil were used as control. A solvent control was also set up with media and solvent. The experiments were done in triplicates.

2.4 Statistical analysis

All statistical analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was performed on all experimental data and means were compared using Duncan's multirange test. The significance level was $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Antifungal Activity of Six Plant Essential Oils against Phytopathogenic Fungi *Aspergillus niger*

The antifungal activity of essential oils was studied using growth inhibition assay or poisoned food technique. Essential oils were added in different concentration 0.1, 0.25, 0.5, 0.75, 1, 5, and 10 % in the media. The results of the antifungal tests revealed that the essential oil treated media inhibited the mycelial growth at varying levels. Effect of Lemongrass, Orange, Mentha, Turmeric, Eucalyptus and clove oil is shown in Table 1, 2, 3, 4, 5 and 6 respectively.

3.1.1 Lemongrass oil

Lemon grass oil was found to be most effective. The growth of the fungus was inhibited by all the concentrations (0.1- 5 %) of lemon grass oil. The control plates showed rich growth in a week (Table 1). The presence of active component citral in lemon grass oil might be responsible for its antifungal activity. Similar results were reported where biological activity of Lemongrass and peppermint essential oil volatiles was tested against *Aspergillus* spp. Lemongrass and peppermint essential oil vapours produces a fungitoxic effect by causings reduction in conidition, loss of pigmentation and disrupted conidispore structure [16].

Table 1: Effect of Lemon grass oil on the growth of *A. niger*

Lemon grass oil conc. (%) in media	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.1	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.25	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.75	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
1	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
2.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0a	0±0a
Control	1.6±0.2 c	2.03±0.06 b	3.17±0.29 b	5.77±0.32 b	6.73±0.31 b	8.23±0.25 b	9.00±0 b
Solvent control	1.27±0.12 b	2.3±0.1 b	3.23±0.21 b	5.27±0.31 b	6.33±0.58 b	8.33±0.58 b	9.00±0 b

Mean± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column not sharing a common letter differ significantly (p<0.05) from each other (Duncan's multirange test)

3.1.2 Orange oil

Orange oil was not effective against the fungus. Only the higher concentrations (2.5 and 5 %) were able to restrict the fungal growth. Control plates showed abundant growth (Table 2).

Table 2: Effect of Orange oil on the growth of *A. niger*

Orange oil conc. (%) in media	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.1	1.83±0.66 d	2.4±0.1d	2.73±0.12 e	4.03±0.15 d	6.57±0.21 e	8.0±0 e	9.0±0 e
0.25	1.6±0.17 c	1.8±0.1c	2.1±0.26 d	2.73±0.31 c	2.83±0.15 c	3.97±0.06 d	5.4±0.2 d
0.5	1.43±0.12 c	1.57±0.21 c	1.73±0.15 c	1.97±0.06 b	2.4±0.1 b	2.97±0.15 c	3.57±0.15 c
0.75	1.3±0.2 c	1.3±0.1 b	1.4±0.1 b	1.8±0.2 b	2.3±0.15 b	2.77±0.15 b	3.07±0.12 b
1	0.55±0.5 b	1±0 b	1.13±0.06 b	1.5±0.1b	2.1±0.2 b	2.5±0.1 b	3±0.1 b
2.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
Control	1.6±0.2 c	2.47±0.32 d	3.63±0.51 f	5.97±0.00 e	6.73±0.64 e	8.17±0.29 e	9.00±0 e
Solvent control	1.33±0.12 c	2.73±0.31 d	3.7±0.26 f	5.2±0.2 e	6.6±0.66 e	8.37±0.35 e	9.00±0 e

Mean± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column not sharing a common letter differ significantly (p<0.05) from each other (Duncan's multirange test)

3.1.3 Mentha oil

Mentha oil also showed similar results as lemongrass. All the concentrations ranging from 0.1-5% restricted the growth of *A. niger* during the test period (Table 3). In comparison, control showed excellent growth. The bioactive compound menthol in mentha oil may be causing the bioactivity. The 5 ppm concentration of essential oil of *M. piperita* completely inhibited the mycelial growth of *Aspergillus niger* to the same extent as 5 ppm of Ketoconazole (standard antibiotic) [34]. *M. piperita* EO has been shown to cause inhibitory effects against radial fungal growth and aflatoxin production by *Aspergillus* species [35].

Table 3: Effect of Mentha oil on the growth of *A. niger*

Mentha oil conc. (%) in media	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.1	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.25	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.75	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
1	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
2.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
Control	1.67±0.31c	2.03±0.06 c	3.4±0.69 c	5.5±0.5 c	6.6±0.53 c	7.87±0.23 c	9.00±0 c
Solvent control	1.33±0.32 c	2.47±0.31 d	3.73±0.38 c	5.3±0.36 c	6.5±0.5 c	8.4±0.4 d	9.00±0 c

Mean± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column not sharing a common letter differ significantly (p<0.05) from each other (Duncan's multirange test)

3.1.4 Turmeric oil

Growth of *A. niger* was not inhibited with turmeric essential oil. It did not show any growth inhibition till the concentration reached 2.5 % and 5 %. Concentrations from 0.1 -1 % allowed growth of fungus (Table 4).

Table 4: Effect of Turmeric oil on the growth of *A. niger*

Turmeric oil conc. (%) in media	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.1	1.6±0.17 c	2.57±0.21d	2.83±0.25 c	3.4±0.26 e	4.47±0.31 d	5.7±0.26 e	9.00±0 e
0.25	1.5±0.1c	1.73±0.15 c	2±0 c	2.57±0.15 d	2.9±0.44 c	3.7±0.36 d	4.33±0.32 d
0.5	1.33±0.21b	1.63±0.21 c	1. 7±0.26 b	2.03±0.06 c	2.17±0.21 b	3.20±0.17 c	3.53±0.15 c
0.75	1.03±0.06 b	1.13±0.06 b	1.37±0.15 b	1.80±0.1 b	2.23±0.15 b	2.53±0.12 b	3.17±0.15 b
1	0.67±0.29 b	0.93±0.12 b	1.27±0.06 b	1.7±0.6 b	2.2±0.2 b	2.4±0.3 b	2.9±0.3 b
2.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
Control	1.53±0.06 c	2.13±0.06 d	3.3±0.15 d	5.1±0.26 f	6.73±0.64 e	8.17±0.29 f	9.00±0 e
Solvent Control	1.5±0.1 c	2.37±0.38 d	3.3±0.2 d	5±0.2 f	6±0.2 e	8.07±0.06 f	8.7±0.3 e

Mean± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column not sharing a common letter differ significantly (p<0.05) from each other (Duncan's multirange test)

3.1.5 Eucalyptus oil

Eucalyptus oil was also effective like lemongrass and mentha oil. The concentrations 0.1- 5 % showed luxuriant growth of fungal mycelium (Table 5). Growth was observed in control and solvent control plates. Cineol in eucalyptus oil might be inhibiting the fungus. The results are in agreement with other workers studying antifungal activity of Eucalyptus *(Eucalyptus camaldulensys* L.) essential oil against six phytopathogenic fungi such as *Penicillium digitatum, Aspergillus flavus, Colletotrichum gloeosporioides, Pythium ultimum, Rhizoctonia solani and Bipolaris sorokiniana*. The results showed complete inhibition of mycelial growth in *Pythium ultimum*, *Rhizoctonia solani* in all concentration of essential oil [36].

Table 5: Effect of Eucalyptus oil on the growth of *A. niger*

Eucalyptus oil conc. in media (%)	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day7
0.1	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.25	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.75	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
1	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
2.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
Control	1.7±0.15 b	2.27±0.38 b	3.57±0.6 b	5.6±0.61 c	6.8±0.35 c	8.03±0.06 b	9±0 b
Solvent Control	1.13±0.06 b	2.3±0.10 b	3.23±0.21 b	5.17±0.25 b	6±0.0 b	8.13±0.23 b	9±0 b

Mean± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column not sharing a common letter differ significantly (p<0.05) from each other (Duncan's multirange test)

3.1.6 Clove oil

Clove oil showed very good inhibition with all the concentrations tested ranging from 0.1-5 %. The control and solvent control plates allowed full growth of fungus (Table 6). The results revealed that clove oil is a strong antifungal agent. Eugenol present in the oil might be responsible for the efficacy. In another study clove oil inhibited the growth of *Aspergillus flavus* [37].

Table 6: Effect of Clove oil on the growth of *A. niger*

Clove oil conc. (%) in media	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.1	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.25	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
0.75	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
1	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
2.5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
5	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0a	0±0a
Control	1.53±0.21b	2.23±0.15b	3.3±0.17 b	5.83±0.21 b	6.8±0.26 b	8.63±0.4 b	9±0 b
Solvent control	1.33±0.32 b	2.33±0.32 b	3.47±0.21 b	5.63±0.32 b	6.9±0.17 b	8.67±0.58 b	9±0 b

Mean± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column not sharing a common letter differ significantly ($p<0.05$) from each other (Duncan's multirange test)

4. CONCLUSION

In this study six plant essential oils were investigated against phytopathogenic fungus, *A. niger*. Growth inhibition studies indicated that clove oil, lemongrass, eucalyptus and mentha oil were found to be potent against the fungus. They completely inhibited the growth of fungus in all the concentrations tested. However, turmeric and orange oil were not effective. Hence it is suggested that clove oil, lemongrass, eucalyptus and mentha oil may be used as natural antifungal agents against *A. niger*. These may be further tested in field conditions and formulated to be used as an environmentally safe alternative to chemical fungicides.

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