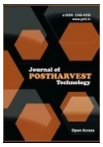

Journal of Postharvest Technology
2022, **10**(3): 113-124
www.jpht.in

RESEARCH ARTICLE

Antifungal activity of seven essential oils against storage fungi *Aspergillus flavus*

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Received: 26.05.2022 Accepted: 22.07.2022

ABSTRACT

Several fungi cause diseases in crop plants. Of these some fungi predominates in agricultural produce during post-harvest storage. Storage fungi can grow at very low moisture range. During storage they produce certain toxic metabolites in the form of mycotoxins in the stored commodities making them unfit for human consumption. Aflatoxin is a mycotoxin produced by *Aspergillus flavus*, which is carcinogenic. Chemical fungicides are generally used to control the plant diseases. However these fungicides have deleterious effects on human and environment. Alternative sources of fungicides are being searched world over. The present study shows the inhibitory effect of seven essential oils Ajwain, Orange, Deodar, Dalchini, Guava, Jaifal and Clove against *Aspergillus flavus.* Mycelial growth inhibition assay was conducted to test the efficacy of essential oils. Growth inhibition studies showed that Ajwain, Dalchini, Clove, Jaifal and Deodar oils were found to be very effective against the test fungus. However Orange and Guava oil were not effective against the fungus.

Keywords: Storage fungi, *Aspergillus flavus*, essential oils, growth inhibition

Citation: Pandey, R.K. 2022. Antifungal activity of seven essential oils against storage fungi *Aspergillus flavus*. *Journal of Postharvest Technology*, **10**(3): 113-124.

INTRODUCTION

Aspergillus flavus is a storage fungi. A storage fungi generally predominates in agricultural produce during post-harvest storage. These fungi grow at moisture level in range of 13 to 18%. There are a number of storage fungi such as species of *Aspergillus, Penicillium, Alternaria, Fusarium, Curvularia, Cladosporium, Rhizopus, Mucor* and *Syncephalastrum*. Some of these storage fungi not only bring about deterioration in agricultural produce during storage but they also produce toxic metabolites in the form of mycotoxins in the stored commodities. *Aspergillus flavus* is the most important member of this group. It produces aflatoxins which is extremely toxic. Extreme toxicity of aflatoxins lies in the fact that they are extremely stable and dangerous in minute quantities. Further, once formed they can't be removed away from the commodity concerned by processing or removal of visible mould growth. Aflatoxins are not destroyed by heat because they are heat stable. Aflatoxin is hepato-carcinogenic and damages hereditary material. It can pass from animal feeds to their milk. It is named as aflatoxin B1, B2, G1, G2, M1, M2 and ochratoxin A. M1 and M2 are milk aflatoxins. Continuous consumption of aflatoxin rich food results in their accumulation in body and this accumulation is responsible for its lethal effect. *Aspergillus flavus* can grow on wide variety of substrates such as cereals (wheat, rice and maize), pulses (peanut, pea and gram), oil seeds (mustard), dry

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ISSN: 2348-4330

fruits, spices, green fruits and vegetables (Bilgrami, 1984). Further if fungus growth occurs, there is always the possibility of aflatoxin production. Although the highest concentrations of aflatoxins are formed in food crops grown and stored in warmer areas of the world, the international trading of important commodities ensure that aflatoxins are not a problem for the producing nations but also of importing countries. Governments of many developed nations carry out surveillance on a regular basis to monitor the intake of aflatoxins by the human population so that the actions can be taken if this becomes necessary. Permissible aflatoxins levels for different food products are fixed by these nations. Since aflatoxins are extremely toxic, these limits are set at very low levels. In developing countries often the good quality products are exported while substandard produce unacceptable to foreign buyers (because they exceed regulatory limits for aflatoxin content) is sold to the domestic market (Dawson, 1991). Therefore in poorer developing countries such contamination has more serious consequences, affecting agricultural economies, reducing annual production and good quality exports and seriously affecting the health of the population.

The complete elimination of aflatoxins in human and animal food, while desirable, is extremely unlikely as they have the potential to arise in a wide range agricultural produce. It is generally accepted that amounts in food should be reduced to rock bottom levels that are technologically possible. To control fungal deterioration of agricultural produce, many organic and inorganic fungicides are developed and used. The use of the many of those has, however, been restricted due to their undesirable side effects like a high and acute toxicity, the long degradation periods, their concentration in food chain, the suspected dangers of chronic poisoning through the continual intake of small quantities (Samson et al., 1995; Kumar et al., 2007). Besides, on account of development of newer races of pathogens, many of those fungicides are gradually becoming out of date (Dikshit, 1980). As such the development of newer, effective and harmless fungicides is required on an increasing scale. According to Brandes (1967) much of our efforts are being wasted in routine testing of the quality fungicides, when there's a pressing need to investigate new sources of effective fungicides (Brandies, 1967). Furthermore, the sources of the synthetic fungicides are largely petrochemicals which are exhaustible. Therefore, haunt for inexhaustible sources of such chemotherapeutants is very desirable.

Green plants appear to be the reservoir of effective chemotherapeutants and may provide reversible source of useful pesticides (Swaminathan, 1978). Tropical floras, in contrast to their temperate zone counterparts, have developed a more efficient and varied defense mechanism due to the far severe conditions for survival. They, thus provide an immense and intriguing source for isolating natural secondary plant metabolites, which exhibit interesting antimicrobial properties. Although just a few 15,000 secondary plant metabolites are chemically identified, their total number may exceed 4,000,000 (Saxena, 1993). They are vast cornucopia of defense chemicals. Recent reports on the likelihood of use of higher plants and their constituents have indicated their usefulness in providing fungicides, which are largely non-phytotoxic, more systemic and simply biodegradable (Fawcett and Spencer, 1969; Beye, 1978). They are sustainable and can be continuously propagated year after year and do not have any negative impact on the environment. Further, where plants are used as storage protectants, they are nearly always applied to regulate insect pests. This is reflected in volumes of research directed to identifying insecticidal plants and plant extracts. Nevertheless, some work has been undertaken to work out whether plants can control storage fungi using essential oils. 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 (Burkil et al.,1966). 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 do not have any adverse effects on humans and animals.

The *Trachyspermum ammi* (Apiaceae) commonly known as Ajowan is one of the aromatic seed spice originated from the Middle East, India, Iran, Afghanistan, and Egypt. In these regions, it's traditionally used as a medicinal plant for its antiseptic, appetizer and carminative properties. The main component of ajwain oil is a phenolic compound thymol. Thymol has been reported to be a germicide, antispasmodic and antifungal (Gandomi, 2014).

The *Citrus reticulata* (Rutaceae) peel essential oil (Orange oil) has wide applications in the confectionary, toiletry and perfumery industry. They show antimicrobial properties such as antifungal, antiviral, antibacterial and antiparasite (Soni and Soni, 2014, Rehman et al., 2007). Limonene was found to be the major component (84.2%) (Sharma and Tripathi, 2006).

Cedrus deodara (Pinaceae) volatile oil has great pharmacological activities and demand within the market. *C. deodara* volatile oil has antimicrobial, insecticidal, molluscicidal, germicidal, anti-tubercular activities in pharmacology. *C. deodara* oil has high demand in fragrance industries on account of its specific characteristics. Himalayan *C. deodara* consists of α-Himachalene, β-Himachalene, γ-Himachalene, α and γ atlantone as its main constituents (Chaudhary et al., 2011).

Cinnamomum zeylanicum member of the Lauraceae family that grows wild in India, Sri Lanka, Indo-china, and Madagascar. The inner bark of this plant has been used as a potent therapeutic agent in ethnomedicine and as a flavouring ingredient in foods (Unlu, et al., 2010). Essential oils derived from medicinal herbs have versatile applications in cosmetics, food, beverages, preservation, fragrance, and pharmaceutical industries. These bioactive compounds indicate several positive biological properties, such as antioxidant, antiviral, antibacterial, antifungal, insecticidal, and anticancer activities (Shakeri, et al., 2014). GC-MS analysis resulted in identification of 17 chemical compounds for *C. zeylanicum* volatile oil. (E)-cinnamaldehyde (71.50%), linalool (7.00%), β-caryophyllene (6.40%), eucalyptol (5.40%), and eugenol (4.60%) were the main components of the essential oil.

Psidium guajava is a medicinal plant belongs to the family Myrtaceae (Wagner et al., 1990). Its common name is guajava. Hydro distillation method was used for isolation of oil from the leaves. It gives about 0.45 ml (0.38% v/w) of a yellowish oil of pleasant odor. GC–MS analysis of the oil from the leaves of *P. guajava* was done. This led to the identification of fifty-four compounds which was representing about 98.172% of the total oil. The major chemical constituents detected in the fresh leave oil were iso-caryophyllene (33.53%), veridiflorene (13.00%), farnesene (11.65%), dl-limonene (9.84%), d-cadinene (1.75%), a-copaene (2.80%), a-humulene (3.74%), aromadendene (1.70%) and scadinol (0.08%) (Weli, et al*.,* 2019).

Myristica fragrans Houtt is a flowering vine in the family of Myristiceae cultivated for its fruit, which is usually used as a spice and seasoning. The local name of this plant is nutmeg tree (Jaifal in Hindi). Jaifal is one of the oldest and widely used spice in the world. The nutmeg spice is produced from its unripe but fully developed fruits. The fruit of this plant has the characteristics aroma and flavour due to their chemical substances especially the volatile oil. Fruit is used to produce nutmeg rind and preserve candy, pickle, chutney and powder (Joshi *et al.,* 1996). Jaifal is also used as components of curry powder, teas and soft drinks. It is also mixed in milk and alcohol (Olaleye et al., 2006). Nutmeg contains about 10% essential oil, chiefly composed of terpene hydrocarbons (sabinene and pinene), myrcene, phellandrene, camphene, limonene, terpinene, p-cymene and other terpene derivatives (Jaiswal et al., 2009).

Syzygium aromaticum, commonly known as clove, is a median size tree (8-12 m) from the Myrtaceae family. It is native from the Maluku islands in east Indonesia. Concentrations up to 18% of volatile oil are often found within the clove flower buds. About 89% of the clove essential oil is eugenol, 5% to 15% is eugenol acetate and β-cariofileno (Jirovetz, et al*.,* 2006). α-humulen, β-pinene, limonene, farnesol, benzaldehyde, 2-heptanone and ethyl hexanoate are the other volatile compounds of

the essential oil of clove. *Syzygium aromaticum* (cloves) are found to be particularly effective, often completely inhibiting both fungal growth and toxin production (Hitokoto, et al., 1980; Mabrouk and El-Shayeb, 1980).

The objective of this study was to examine the antifungal activity of seven plant essential oils from *Trachyspermum ammi* (Ajwain seed oil), *Citrus reticulata* (Orange peel oil), *Cedrus deodara* (Deodar heart wood oil), *Cinnamomum zeylanica* (Dalchini bark oil), *Psidium guajava* (Guajava leaf oil), *Myristica fragrans* (Jaifal seed oil) and *Syzygium aromaticum* (Clove flower bud oil) against storage fungi *Aspergillus flavus.*

MATERIALS AND METHODS

Test fungus

Aspergillus flavus 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.

Essential oils

Plant essential oils from *Trachyspermum ammi* (Ajwain seed oil), *Citrus reticulata* (Orange peel oil), *Cedrus deodara* (Deodar heart wood oil), *Cinnamomum zeylanica* (Dalchini bark oil), *Psidium guajava* (Guajava leaf oil), *Myristica fragrans* (Jaifal seed oil) and *Syzygium aromaticum* (Clove flower bud oil) were extracted. The essential oil were extracted from the different parts of the plants such as leaves, stems, roots, fruit peels, fruits, woods etc using Clevenger's apparatus (Clevenger, 1928). This apparatus extract the volatile fraction from the plant parts through hydro-distillation method. Two distinct fractions comprising an upper oily layer and a lower aqueous layer were obtained which were separated by carefully regulating the stopper of the apparatus. The upper oily layer was made anhydrous by treating it with 0.5 grams of anhydrous sodium sulphate in order to obtain the pure essential oil.

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 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 µl/ml 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. flavus* were inoculated 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.

Statistical analysis

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

Results and Discussion

The antifungal activity of the essential oils was measured using poisoned food technique. Different concentrations of essential oils 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 µl/ml were added into the medium. 20 ml of poisoned media was poured in petriplate. The results of the antifungal test revealed that essential oil treated medium inhibited the mycelia growth at varying levels. The results of ajwain oil, orange oil, deodar oil, dalchini oil, guajava oil, jaifal oil and clove oil is shown in Table 1, 2, 3, 4, 5, 6 and 7, respectively.

Ajwain Oil

Ajwain essential oil is very effective. It inhibits the mycelia grow completely from 1.0-5.0 µl/ml concentrations. But at 0.5 µl/ml concentration mycelial growth is visible. The control plate showed rich growth in a week (Table 1). Thymol present in the ajwain essential oil is responsible for its antifungal activity. Similar results were reported by other studies where ajwain oil was tested against *Aspergillus spp.* Ajwain oil also inhibited the growth of fungi by vapour action also by inhibiting conidial germination.

Table1: Effect of Ajwain seed oil on the growth of *Aspergillus flavus*

Ajwain seed oil conc. in medium (µl/ml)	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.5	0.78±0.68	1.83±0.46	2.32±0.44	3.45±0.86	5.11±0.22	6.01±0.74	6.42±0.56
1.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Control	1.73±0.66	2.21±0.01	4.58±0.01	5.45±0.00	6.76±0.00	8.83±0.01	9.00±0.00
Solvent Control	1.77±0.05	2.33±0.02	4.61±0.00	5.62±0.02	6.79±0.01	8.88±0.04	9.00±0.00

Mean ± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column. Means were compared using Duncan's multirange test. The Significance level was p<0.05.

Orange oil

Orange oil is not very effective in inhibiting the mycelia growth of the fungus. Essential oil from 0.5 to 4.5 µl/ml was not able to inhibit the mycelial growth. This oil inhibits the growth only at higher concentrations (5.0 µl/ml and above). Limonene was the

most abundant compound present in the oil may be responsible for inhibitory activity of the oil. The control plate showed abundant growth (Table 2).

Table 2: Effect of Orange peel oil on the growth of *Aspergillus flavus*

Orange peel oil conc. in medium (µl/ml)	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.5	1.78±0.66	2.22±0.71	5.11±0.02	5.58±0.04	7.21±0.06	8.86±0.73	8.95±0.76
1.0	1.67±0.05	1.73±0.32	5.51±0.44	5.55±0.07	6.46±0.54	7.22±0.03	8.69±0.89
1.5	1.61±0.02	1.69±0.66	4.00±0.06	4.22±0.05	5.47±0.66	6.06±0.66	6.77±0.09
2.0	1.53±0.01	1.55±070	3.67±0.52	3.75±0.64	4.82±0.01	5.22±0.71	6.11±0.06
2.5	1.48±0.66	1.51±0.45	3.23±0.61	3.55±0.73	4.65±0.44	5.00±0.64	5.58±0.33
3.0	1.45±0.02	1.49±0.03	2.89±0.01	2.99±0.83	3.11±0.56	4.82±0.88	5.00±0.92
3.5	1.21±0.00	1.32±0.01	1.99±0.88	2.00±0.22	2.40±0.41	3.10±0.11	3.25±0.75
4.0	1.00±0.55	1.10±0.22	1.00±0.44	1.20±0.09	1.01±0.04	1.07±0.66	1.21±0.98
4.5	0.50±0.40	0.58±0.06	0.60±0.77	0.62±0.52	0.70±0.61	0.75±0.45	0.80±0.56
5.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Control	1.75±0.00	2.21±0.01	4.58±0.01	5.45±0.00	6.76±0.00	8.83±0.01	9.00±0.00
Solvent Control	1.67±0.05	2.33±0.02	4.61±0.00	5.62±0.02	6.79±0.01	8.88±0.04	9.00±0.00

Mean ± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column. Means were compared using Duncan's multirange test. The Significance level was p<0.05.

Deodar oil

Deodar oil was very effective in inhibiting the growth of the test fungi. It completely inhibited *A. flavus* from 3.0-5.0 µl/ml concentrations. At lower concentrations from 0.5-2.5 µl/ml it was less effective in inhibiting the growth of fungi. In its comparison control plate showed abundant growth (Table 3). Himachalene, the major component of this oil is responsible for its inhibitory action. This oil also inhibits conidial germination by its vapour action.

Table 3: Effect of Deodar heart wood oil on the growth of *Aspergillus flavus*

Deodar heart wood oil conc. in medium (µl/ml)	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.5	1.62±0.61	2.58±0.25	4.45±0.56	5.42±0.66	6.22±0.56	6.98±0.42	7.22±0.65
1.0	1.48±0.44	2.45±0.53	4.15±0.46	5.55±0.22	5.89±0.68	6.10±0.22	6.32±0.40
1.5	1.21±0.23	2.15±0.32	3.64±0.34	4.64±0.44	4.98±0.46	5.05±0.64	5.15±0.20
2.0	0.50±0.33	1.87±0.45	2.48±0.36	3.49±0.63	3.83±0.35	4.50±0.56	4.66±0.49
2.5	0.50±0.11	1.65±0.21	2.11±0.31	3.24±0.22	3.45±0.54	4.23±0.44	4.22±0.36
3.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

3.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Control	1.59±0.98	2.78±0.66	4.79±0.87	5.88±0.77	6.99±0.79	8.89±0.43	9.00±0.00
Solvent Control	1.58±0.85	2.74±0.55	4.69±0.98	5.72±0.33	6.98±0.32	8.89±0.44	9.00±0.00

Mean ± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column. Means were compared using Duncan's multirange test. The Significance level was p<0.05.

Dalchini oil

The minimum inhibitory concentration of *Cinnamomum zeylanicum* bark oil was found to be 1.0 µl/ml. From 1.0-5.0 µl/ml there was complete inhibition of growth of *Aspergillus flavus*, the test pathogen. At 0.5 µl/ml signs of growth were observed in the petriplates. Control plate showed abundant growth (Table 4). The essential oil has an intense suppressing effect on the fungal spore germination also. This spore germination inhibition was caused in dosage response manner. Velluti et al. (2003) reported that the eugenol, present in the *Cinnamomum zeylanicum* bark oil is responsible for its antimicrobial activity. This activity is attributed to the presence of an aromatic nucleus and a phenolic OH group. The OH group is very reactive and form hydrogen bonds with active sites of target enzymes. Generally, the antimicrobial activity of an essential oil is attributed to its major components, the synergistic or antagonistic effect of compounds in minor percentage in the mixture has also to be considered (Daferera et al., 2003, Souza et al., 2007).

Table 4: Effect of Dalchini bark oil on the growth of *Aspergillus flavus*

Dalchini bark oil conc. in medium (µl/ml)	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.5	0.89±0.56	1.44±0.52	3.20±0.55	4.46±0.66	4.78±0.63	5.75±0.46	6.11±0.52
1.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Control	1.50±0.00	2.21±0.01	4.58±0.01	5.45±0.00	6.76±0.00	8.83±0.01	9.00±0.00
Solvent Control	1.57±0.05	2.33±0.02	4.61±0.00	5.62±0.02	6.79±0.01	8.88±0.04	9.00±0.00

Mean ± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column. Means were compared using Duncan's multirange test. The Significance level was p<0.05.

Guava oil

Psidium guajava (Myrtacee) leaf essential oil is relatively less effective in inhibiting the mycelia growth of *Aspergillus flavus*. It did not show any growth inhibition till the concentration reached at 5.0 µl/ml and above. Concentrations from 0.5 to 4.5 µl/ml allowed the growth of fungus (Table 5).

Jaifal oil

Jaifal (*Myristica fragrans*) oil inhibited the growth of the test fungi completely at 2.0 µl/ml concentration. It could not inhibit the mycelial growth of the *Aspergillus flavus* from concentrations 0.5-1.5 µl/ml. Pande et al. (2010) found that essential oil of *Myristica fragrans* to be fungistatatic. Even at a dose as high as 15000 ppm it showed fungistatic nature. Control plates showed the abundant growth of the fungus (Table 6).

Table 5: Effect of Guava leaf oil on the growth of *Aspergillus flavus*

Guava leaf oil conc. in medium (µl/ml)	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.5	1.49±0.52	2.23±0.48	3.32±0.50	4.89±0.34	5.44±0.67	6.32±0.44	7.28±0.37
1.0	1.45±0.46	2.10±0.09	3.11±0.06	4.55±0.67	5.10±0.45	5.48±0.63	6.45±0.42
1.5	1.36±0.54	2.00±0.11	3.00±0.56	4.11±0.34	4.78±0.07	5.11±0.52	5.35±0.45
2.0	1.23±0.22	1.87±0.06	2.89±0.44	3.65±0.08	4.00±0.61	4.89±0.61	5.10±0.61
2.5	1.15±0.00	1.77±0.05	2.71±0.62	3.00±0.42	3.75±0.55	4.23±0.85	4.88±0.22
3.0	1.10±0.36	1.65±0.44	2.52±0.08	2.78±0.76	3.15±0.42	3.87±0.73	4.25±0.63
3.5	1.00±0.44	1.51±0.21	2.25±0.42	2.65±0.65	2.82±0.56	3.44±0.22	3.75±0.44
4.0	0.90±0.21	1.32±0.08	2.10±0.06	2.35±0.41	2.58±0.46	2.96±0.56	3.55±0.66
4.5	0.70±0.38	1.10±0.33	1.56±0.47	1.86±0.22	2.00±0.47	2.11±0.00	2.64±0.53
5.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Control	1.5±0.00	2.21±0.01	4.58±0.01	5.45±0.00	6.76±0.00	8.83±0.01	9.00±0.00
Solvent Control	1.57±0.05	2.33±0.02	4.61±0.00	5.62±0.02	6.79±0.01	8.88±0.04	9.00±0.00

Mean ± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column. Means were compared using Duncan's multirange test. The Significance level was p<0.05.

Table 6: Effect of Jaifal seed oil on the growth of *Aspergillus flavus*

Jaifal seed oil conc. in medium (µl/ml)	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.5	1.53±0.38	2.19±0.64	3.21±0.43	4.79±0.60	5.24±0.54	6.45±0.73	7.10±0.42
1.0	1.32±0.60	1.88±0.39	2.30±0.68	3.55±0.47	4.54±0.65	4.87±0.65	5.22±0.65
1.5	0.67±0.06	0.75±0.22	0.87±0.47	0.91±0.35	1.00±0.48	1.52±0.36	2.10±0.35
2.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

2.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Control	1.50±0.00	2.21±0.01	4.58±0.01	5.45±0.00	6.76±0.00	8.83±0.01	9.00±0.00
Solvent Control	1.57±0.05	2.33±0.02	4.61±0.00	5.62±0.02	6.79±0.01	8.88±0.04	9.00±0.00

Mean ± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column.
Means were compared using Duncan's multirange test. The Significance level was p<0.05.

Clove oil

Clove oil was very effective in inhibiting the growth of *Aspergillus flavus*. It inhibited the growth of the fungus ranging from concentrations 1.5 to 5.0 µl/ml. At lower concentrations ranging from 0.5 to 1.0 µl/ml it showed the signs of growth in the petriplates. Eugenol present in the oil may be responsible for antifungal activity. The control and solvent control plates allowed full growth of the fungus (Table 7).

Table 7: Effect of Clove flower bud oil on the growth of *Aspergillus flavus*

Clove flower bud oil conc. in medium (µl/ml)	Colony diameter (cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.5	1.51±0.64	2.05±0.48	3.98±0.60	4.50±0.36	5.54±0.74	6.46±0.56	6.89±0.46
1.0	0.54±0.60	0.65±0.62	0.89±0.44	1.25±0.63	1.50±0.51	1.87±0.65	1.98±0.74
1.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Control	1.50±0.00	2.21±0.01	4.58±0.01	5.45±0.00	6.76±0.00	8.83±0.01	9.00±0.00
Solvent Control	1.57±0.05	2.33±0.02	4.61±0.00	5.62±0.02	6.79±0.01	8.88±0.04	9.00±0.00

Mean ± Standard Deviation (SD) on three determinations, Values (means of 3 replicates) in each column.
Means were compared using Duncan's multirange test. The Significance level was p<0.05.

CONCLUSION

In present study seven essential oils were investigated against the storage fungi *Aspergillus flavus*. Growth inhibition studies indicated that the essential oils of Ajwain, Dalchini, Clove, Jaifal and Cedrus completely inhibited mycelial growth at concentration as low as 1.0 µl/ml, 1.0 µl/ml, 1.5 µl/ml, 2.0 µl/ml and 3.0 µl/ml respectively. The essential oils of Orange and Guava showed complete inhibition only at higher concentrations and are relatively less effective. Hence it is suggested that Ajwain, Dalchini, Clove, Jaifal and Cedrus oils can be used as natural antifungal agents against *A. flavus.* These oils can be further tested in field conditions and formulated to be environmentally safe alternative to chemical fungicides.

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