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Study on Antifungal Effect of Herbal Compounds against Mycotoxin Producing Fungi

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Abstract Mycotoxins are toxic substances produced mostly as secondary metabolites by filamentous fungi that grown on seeds, grains, feed and contaminate them during storage and pose the most serious threads to human and animals health while consumption. The most alternative aspects derived from previous studies suggested that herbal compounds and plants extracts could act as antifungal and antimycotoxigenic agents without side effects. In the present study the antifungal activity of some herbal compounds and plants extracts from sixteen medicinal plants at different concentration were tested against three important mycotoxin producing fungal species, *Aspergillus parasiticus, Penicillium verucossum, Fusarium monilliforme.* Among the herbal compounds and plant extracts were screened, clove oil at 0.5% concentration was showed (100%) complete inhibition against *A.parasiticus, P. verrucossum, F.monilliforme.* In addition, pepper (0.5%), turmeric (0.5%), eucalyptus oil (2%) was showed (100%) complete inhibition against *Penicillium verucossum.* While all the herbal compounds tested were affected the *Aspergillus parasiticus* growth, garlic and neem oil (2%) have shown high antifungal activity. Jeera, eucalyptus oil, asafetida and karanj oil have shown moderate antifungal activity against *F.monilliforme.* Further, fenugreek showed poor activity against all tested organisms. Thus, the present study proven antifungal activity and prevention of mycotoxin production by selected herbal compounds and plant extracts, which might be recommended as natural preservatives and prevent fungal contamination during storage of cereals, seeds, grains, and feeds.

Keywords: Mycotoxin, Antifungal activity, Herbal compounds, *Aspergillus parasiticus, Fusarium moniliforme, Penicillium verucossum*.

Introduction

Mycotoxins are secondary metabolites of filamentous fungi, produced mainly by five fungal genera namely *Aspergillus, Penicillium, Fusarium, Altenaria* and *Claviceps*. Although hundreds of fungal toxins are known, a limited number of toxins are generally considered to play important roles in

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food safety. They are well known for their health-hazardous effects in human beings and animals (Bullerman, 1979). Around a quarter century back itself, the World Health Organization estimated that approximately 25% of the world's grains and crops were contaminated by mycotoxins, and more than 300 fungal metabolites are reported to be toxic to man and animals (Galvano *et al*., 2001).

Aflatoxins, fuminosins, trichothecenes, ocharatoxin A, cyclopiazonic acid, zearalenone, deoxyvalenol, citrinin, gliotoxin and sterigmatocystin are the major mycotoxins reported and are known to be extremely toxic and cancerous (Reddy *et al*., 2010). The main toxic effects are genotoxicity, terratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immune suppression (Lacey, 1988; Desjardins *et al*., 2000). At cellular level, mycotoxins react with nucleic acids and inhibit the biosynthesis of macromolecules DNA, RNA and protein also act on structure and functions of biological membranes there by impair the energy metabolism (Wang, 1999; Diaz, 2005).

Mycotoxin production is unavoidable and at times unpredictable, which makes it a unique challenge to food safety. They can also be passed along in the food chain and contaminate milk, meat and eggs, posing a greater danger to the health of humans and also the quality regulations of animal products. Decontamination of mycotoxin-contaminated food is not fully successful, and control of mycotoxins is the need of the hour. The development of integrated management strategies is therefore essential to ensure food safety. The development of safer antifungal agents, from plant products are recognized as one of the most promising alternative strategy (Varma and Dubey, 2001).

In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999). Various reports on medicinal plants extracts have shown inhibitory effects against phytopathogenic fungi in vitro (Senhaji *et al*., 2005; Pak *et al*., 2006; Oyedeji *et al*., 2011). Plants contain several phytochemicals including vitamins, alkaloids, flavonoids, terpenoids, carotenoids, coumarins etc., which are known to play important role in defense against bacteria, fungi, herbivores, insects and viruses (Duke and Bogenschutz-Godwi, 1999). Due to their biodegradability and low toxicity, they were of great importance in food industry and offer the possibility to substitute natural for synthetic preservatives and other products (Gurdip Singh and Sumitra Mayurya, 2005). The mechanisms thought to be responsible for toxicity against fungi may involve various targets: interference with the synthesis of cellular walls, alteration of cell permeability, interference with the transport of electron, the nutrient absorption, the adenosine triphosphatase and other metabolic processes of the cell, deactivation of various cellular enzymes and denaturation of cellular

proteins (Marjorie, 1996; Cowan, 1999; Feng and Zheng, 2007; Al-Amiery *et al.,* 2012). Currently, there is little evidence on the antimicrobial properties of the medicinal plants under investigation against phyto pathogen fungi. Hence, the aim of this study was to screen the effects of different spices, essential oils and herbal extracts on growth inhibition level on mycotoxin producing *Aspergillus parasiticaus, Fusarium moniliforme* and *Penicillium verrucosum* fungus.

Materials and methods:

Collection of samples

Herbal compound/spices and plants

Sixteen samples of different herbal compounds/spices and plants, known for their medicinal value in traditional medicine were selected for the study. The list of herbal compounds/spices and plants, the family to which they belong and parts used for antifungal activity is presented (Table 1).

Table 1. Herbal Compounds/Spices and Plant extracts used for screening

S. no.	Common name	Scientific name	Parts Used of plant
1.	Turmeric	*Curcuma longa*	Root
2.	Jerra (Cumin)	*Cuminum cyminum*	Seed
3.	Cinnamon	*Cinnamon zeylanicum*	Stem bark
4.	Asafoetida	*Azafoetida indica*	Stem bark
5.	Black pepper	*Piper nigrum*	Dried fruit
6.	Fenugreek	*Trigonella foenum graceam*	Seeds
7.	Neem oil	*Azadirachta indica*	Seed oil
8.	Karanj oil	*Derris indica*	Oil
9.	Eucalyptus oil	*Eucalyptus globules*	Seed
10.	Clove oil	*Eugenol*	Flower buds
11.	Coriander	*Coriandrum cassia*	Seed
12.	Onion	*Allium cepal*	Bulb
13.	Ginger	*Zingiber officinale roscoe*	Fleshy rhizome
14.	Thulasi	*Ocimum basilicum*	Leaves
15.	Lemon juice	*Citrus lemon*	Fruit peel
16.	Garlic	*Allium sativam*	Bulb

Processing of samples

The herbal compounds/spices (turmeric, jeera, cinnamon, asafetida, black pepper, cardamom, coriander, and fenugreek) and plants (onion, garlic, ginger, lemon, thulasi) were obtained from the local market and immediately kept in refrigerator until starting the experiments. After sterilization samples were dried in hot air oven for 1-2 days at 50 ℃, ground to powder form in warring blender and sieved with 1 mm mesh. These processed samples and karanj oil, clove oil, eucalyptus oil and neem oil were added to the growth media of *Aspergillus parasiticus* and *Fusarium moniliforme* separately in 250 ml Erlenmeyer flasks containing freshly prepared 100 ml sterile potato dextrose agar (PDA), and also *Penicillium verrucosum* separately in 250 ml Erlenmeyer flasks containing freshly prepared 100 ml sterile Czapek yeast extract agar (CYEA). The concentration obtained was 0.5, 1.0, and 2.0% for each sample respectively.

Fungal strains

The pathogenic fungal strains as *Aspergillus parasiticus* (NRRL 2999), *Penicillium verrucosum (*MTCC 1758) and *Fusarium moniliforme* (MTCC 156) were purchased from National Institute of Animal Nutrition And Physiology (NIANP), Bangalore.

Antifungal activity

All the processed samples were thoroughly mixed with the medium (PDA) after autoclaving, then media (100 ml) was transferred into 9 cm petri dishes, each in triplicate and then cooled. The media without tested samples served as control. After complete solidification of the medium to these petri dishes, 0.1 ml diluted spore suspension (25×10^7 CFU / ml) of *Aspergillus* parasiticus (NRRL 2999), *Penicillium verrucosum* (MTCC 1758) and *Fusarium moniliforme* (MTCC 156) were added, spread, sealed with parafilms and incubated for 7 days at 27–30 ℃ for the control reach of full growth. Anti-fungal properties of the samples were judged by counting the colonies on the 7th day. Fungal growth was measured for each colony and percent inhibition (I %) of the fungal growth was calculated according to the following formula:

Percent inhibition = (Growth in control-Growth in treatment/Growth in control) x 100

Statistical analysis

Colony fungal growth and antifungal activity data were evaluated by analysis of variance (ANOVA). The mean values were tested for all significance difference by Duncan's Multiple Range Test (DMRT) as the mean ± S.D and p values < 0.001 were considered statistically significant.

Results and Discussion

Excessive usage of pesticides in agriculture to overcome the pre-harvest and post-harvest problem was resulted in many toxic epidemics. Generally, toxic synthetic fungicides are not exploited to prevent biodeterioration of grains during storage even though they are exploited for improving seed quality (Harris *et al*., 2001). Thus, there is an urgent need to search for alternative method for prevention of biodeterioration of grains during storage without any toxicity to the consumer. Many higher plants produce economically important organic compounds, pharmaceuticals and pesticides. Hamburger and Hostettmann (1991) reported that the total number of plant chemicals may exceed 400,000 and out of it more than 10,000 are secondary metabolites whose major role in plant is defensive in nature. Thus, plant based secondary metabolites, which have defensive role may be exploited for the management of storage pest. However, the most species of higher plants have biologically active constituents which might be used as new sources of commercially valuable pesticides (Balandrin *et al*., 1985). These biologically active plant derived fungicides are expected to play an increasingly significant role against pathogenic fungi. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for protection of plant products and will have a prominent role in the development of future commercial antifungal agents for protection strategies, with special reference to the management of fungal contamination (Varma and Dubey, 1999; Gottlieb *et al*., 2002). This study was made to contribute to clear the lack of information on the screening/evaluation of diverse plants for their antifungal potential. In the present study the inhibition of fungal growth in *Aspergillus parasiticus, Penicllium verucosum, and Fusarium moniliforme* were evaluated. The anti-fungal activity of some herbal compounds/spices and plants extracts at different concentrations were evaluated.

Spices and herbs have been used as food additives since ancient times, as flavoring agents as well as natural food preservatives. A number of spices showed anti microbial activity against different type of microorganism. Anti microbial activity depends on the type of spices or herbs, type of food and

microorganism, as well as on the chemical composition and content of extracts and essential oils. Various reports on medicinal plant extracts have shown inhibitory effects against phyto pathogenic fungi in vitro (Senhaji *et al*., 2005; Pak *et al*., 2006; Oyedeji *et al*., 2011). Our present results corroborate with these findings.

Among the herbal compounds/spices screened against fungal growth, clove oil (0.5 %) showed complete inhibition of *Aspergillus parasticus* growth (Fig. 1a, 1b). Garlic, neem oil, karanj oil, turmeric powder, asafetida powder (0.5-2%) were represented high antifungal activity versus control (70-75%). Further, herbal compounds such as eucalyptus, jeera, cardamom, pepper, ginger, coriander, lemon, fenugreek and thulasi have showed moderate antifungal activity with ranges between (60-65%). Onion has showed poor antifungal activity even in higher concentration (2%).

The list of herbal compounds/spices screened against *Penicillium verucossum* fungal growth, clove oil (0.5-2%), pepper (0.5-2%), turmeric (2%), eucalyptus oil (2%) were showed complete inhibit growth of *Penicillium verucossum* (Fig. 2a, 2b). While Karanj oil, neem oil, ginger and asafetida were represented high antifungal activity versus control (70-95%) garlic, thulasi, jeera and lemon were moderate (60-70%) in preventing fungal growth against *Penicillium verucossum*. Cardamom, fenugreek, onion and coriander had showed poor anti fungal activity against *Penicillium verucossum.*

Amongst the herbal compounds/spices screened against fungal growth, clove oil showed (0.5%) complete inhibition of *Fusarium moniliforme* (Fig. 3a, 3b) followed by asafetida (75%). Pepper, jeera, turmeric, neem oil, eucalyptus oil, cardamom, karanj oil, lemon have showed moderate antifungal activity ranges between (60-70%) versus control. Garlic, ginger, onion, coriander, fenugreek and thulasi have showed low antifungal activity (50-57%) against *Fusarium moniliforme*.

Previously the anti-fungal activities of some of these compounds were studied by different workers (Bilgrami *et al*., 1992; Gowda *et al*., 2004). They suggested though clove oil is an excellent anti-fungal agent, cost is a major criterion for considering its inclusion in feed. Turmeric powder inhibits sporulation and aflatoxin production, and this effect is due to the presence of anti-oxidant curcumin (Mohan *et al*., 2001). Though, pepper was not strongly anti-fungal against *Fusarium moniliforme* and *Aspergillus parasticus*, but produced tiny colonies, indicating that toxin production could be minimized confirmed through the smaller size of colonies. It has reported that many compounds caused inhibition of aflatoxin B1 production, whereas extracts of plants such as onion and garlic inhibited growth and aflatoxin synthesis (Al-Ghamid, 2001; Prescott *et al*., 2005). Many plant species like neem

(*Azadirachta indica*) were found effective for storage of dry cereals and legumes. Our results of the mycelium growth inhibition assay suggested that clove was most active against all the fungal species selected for the present study. Hence, the present study concludes that, growth of mycotoxin producing moulds as well as toxin production was notably controlled by clove oil, pepper, turmeric and eucalyptus oil in dose dependent manner.

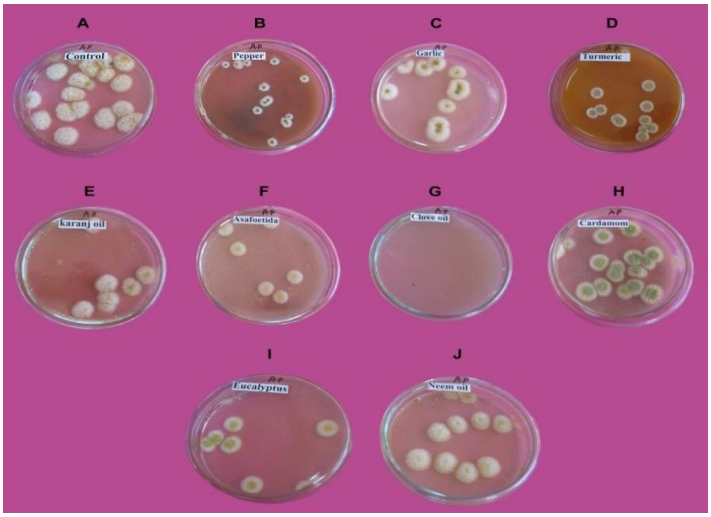

Fig. 1a. Anti-fungal effect of various herbal compounds on *Aspergillus parasiticus*. A-showed control; G-showed complete (100%) inhibition; C, D, E, F & J-showed high (70–75%) antifungal activity; B, H & I-showed moderate (60-65%) antifungal activity

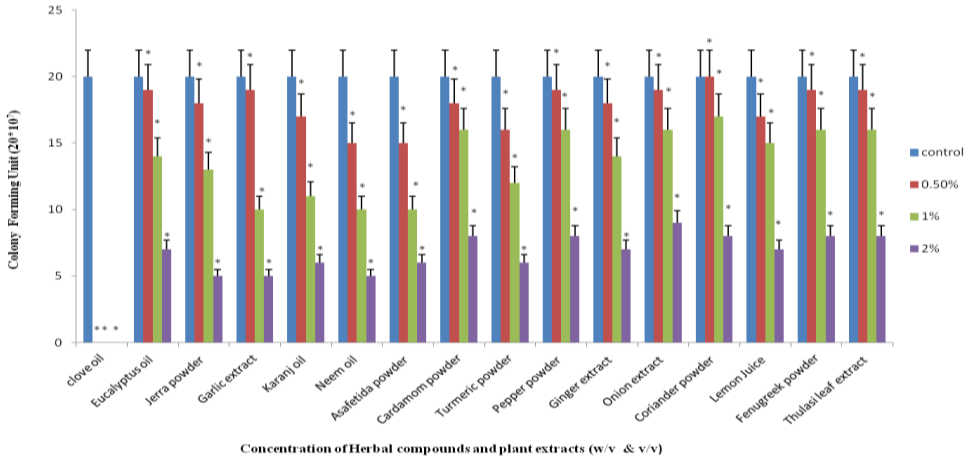

Fig. 1b. Anti-fungal effect of various herbal compounds on *Aspergillus parasiticus*. Data are given as mean values and standard deviations of three replicates for each. Symbols indicate statistically significant when compared with the control group *p <0.01.

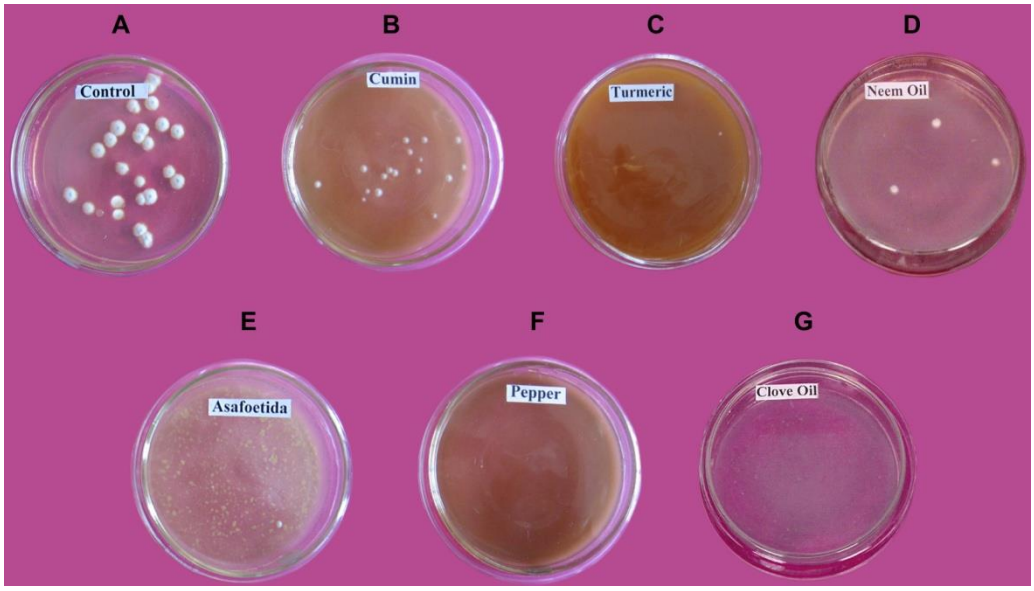

Fig. 2a. Anti-fungal effects of various herbal compounds on *Penicillium verrucosum*. A-showed control; C, F & G-showed complete (100%) inhibition; D & E showed high (70-95%) antifungal activity; B-showed moderate (60-70%) inhibition

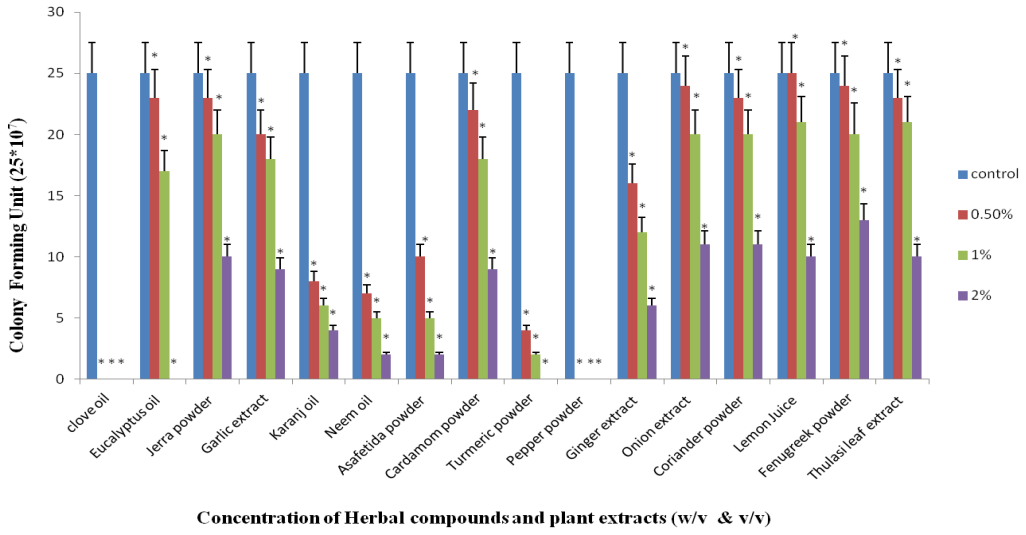

Fig. 2b. Anti-fungal effects of various herbal compounds on *Penicillium verrucosum.* Data are given as mean values and standard deviations of three replicates for each. Symbols indicate statistically significant when compared with the control group *p <0.01

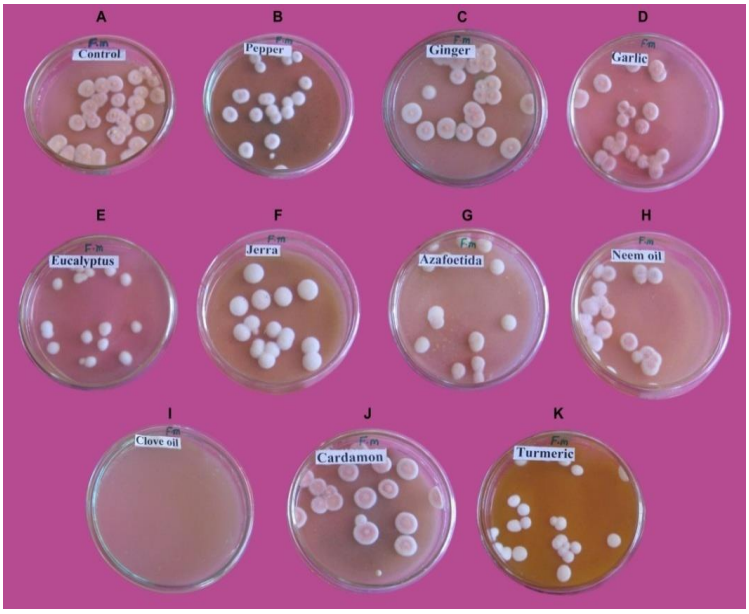

Fig. 3a. Anti-fungal effect of various herbal compounds on *Fusarium moniliforme.* A-showed control; I-showed complete (100%) inhibition; B, E, F, H, J & K-showed moderate (60–75%) antifungal activity; D & C-showed low (50-57%) antifungal activity

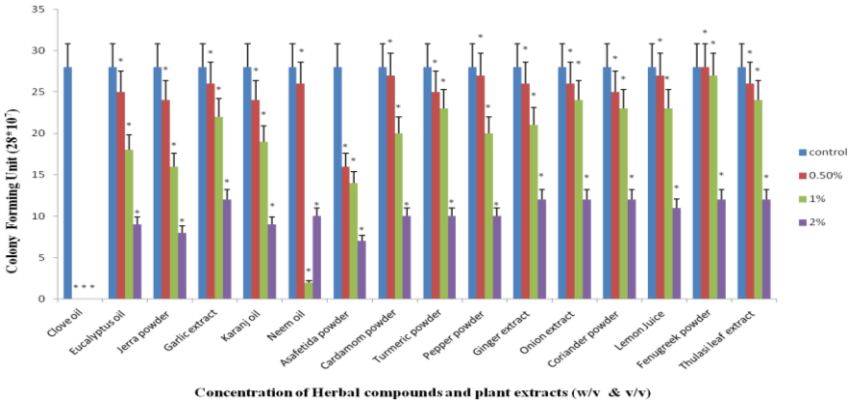

Fig. 3b. Anti-fungal effect of various herbal compounds on *Fusarium moniliforme.* Data are given as mean values and standard deviations of three replicates for each. Symbols indicate statistically significant when compared with the control group *p <0.01

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