Pure Appl. Bio., 1(1): 8-13, June- 2012.

INHIBITION OF AFLATOXIN PRODUCING FUNGUS GROWTH USING CHEMICAL, HERBAL COMPOUNDS/SPICES AND PLANTS

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Abstract

The anti-fungal activity of some chemicals, herbal compounds/spices and plants at different concentrations were evaluated against the toxin producing *Aspergillus flavus* and *Aspergillus parasiticus* growth. Among the chemicals screened, benzoic acid (0.1 – 0.5 %), propionic acid (0.1 – 0.5 %) and copper sulphate (0.2 – 0.5 %) showed complete inhibition of *Aspergillus flavus* growth. In case of the herbal compounds/spices, clove (0.5 %) and clove oil (0.5 %), while among the plants garlic (0.5 %) and onion (0.5 %) showed complete inhibition. All the samples were also screened against *Aspergillus parasiticus* growth. Among the chemicals screened, benzoic acid (0.2 - 0.5 %), copper sulphate (0.1 - 0.5 %) and propionic acid (0.2 - 0.5%) showed complete inhibition of growth. In herbal compounds/spices, clove and clove oil (0.5%), while among plants, garlic and onion (0.5 %) showed complete inhibition of growth. Thus some herbal compounds/spices and some chemicals as preservatives may successfully replace synthetic pesticides and provide an alternative method to protect our staple food from the toxic fungal contamination.

Key words: Chemicals, Herbal compounds/spices, Inhibition, Plants, Toxigenic fungus

Introduction

Mycotoxins have detrimental effects, so a number of strategies have been developed to help prevent the growth of mycotoxigenic fungi as well as to decontaminate and/or detoxify mycotoxin contaminated foods and animal feeds. These strategies include the prevention of mycotoxin contamination, detoxification of mycotoxins present in food and feed and inhibition of mycotoxin absorption in the gastrointestinal tract. The prevention of mycotoxin contamination prior to harvest or during post-harvest and storage is not always possible necessitating decontamination before the use of such materials for food and feed purposes. Therefore various detoxification processes play an important role in helping prevent exposure to the toxic and carcinogenic effect of mycotoxins [1]. Detoxification of mycotoxins is typically achieved by removal or elimination of the contaminated commodities or by inactivation of the toxins present in these commodities by physical, chemical, or biological methods [2]. It is generally accepted that mycotoxin levels in food needs to be reduced as low as technologically possible. Many physical, chemical and biological methods have been applied for the removal and biosynthesis of aflatoxins, and also growth of aflatoxigenic moulds inhibition. However, few of these have practical applications [3, 4].

Many methods have been applied in order to control or inhibit mould growth and maize contamination by aflatoxin, although strategies to control growth of mould and mycotoxin contamination has to be considered [5], for plants products postharvest methods are also recommended [6]. The prevention of mycotoxin production includes all phases of food and feed production, because the mould contamination may occur in the field, during storage, as well as in transport. Adequate storage with optimal temperature and humidity of grains and relative humidity and the hygiene in silos may decrease the growth of toxicogenic moulds[7].

In chemical decontamination methods, treatment by application of ammonia in many raw materials showed high efficacy in order to decontaminate aflatoxins[3]. Several herbal as well as some chemicals materials were tested in order to reduce production of aflatoxin and growth of mould, due to which some herbal compounds and chemicals showed inhibition of fungus Aspergillus growth and reduction in synthesis of aflatoxins. A mushroom extract showed inhibition of the formation of AFB1 -8, 9-epoxide [8]. The application of competitive, non aflatoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* has clearly been shown to reduce the aflatoxin contamination of agricultural commodities such as peanuts, rice, maize and cottonseed mainly through competition for substrate and through the production of inhibitory metabolites [9, 10]. Antipyretic, analgesic and anti-inflammatory properties have reported in *Nigellasativa* [11]. The Kalongi (*Nigellasativa*) crude extracts showed antimicrobial effects on resistant bacteria of different antibiotics [12]. It has been investigated that natural antioxidants treatment for aflatoxin reduction occurred worldwide. These finding verified that the metabolic pathways of microorganisms for aflatoxins was elucidated [13].

The fungitoxic activities have been reported by several extracts of plants, while some of them i.e. cloves, cinnamon, Chinese cassia and thymes along with their mixture showed complete reduction in aflatoxins synthesis [14, 15, 16]. The antipyretic, antitumor, antimalarial and antifungal activities have been reported for different parts of neem tree, neem kernels, extracts of leaf and kernels during storage of grains [17, 18, 19, 20]. To reduce the toxic effect of aflatoxin B1, microorganisms as an antagonist have been used as treatment by biological means [21]. Natural plant extracts and spices are also known to prevent mould growth and mycotoxin production [22, 23, 24]. Specifically extracts from Egyptian plants namely, *Lupinus albus*, *Ammi visnaga* and *Xanthium pungens* have been shown to inhibit the growth of an aflatoxin of A. flavus in a dose-dependent manner [25]. The inhibitory effects exerted by spices and herbs may rely at least in part on phenolic compounds such as coumarins and flavonoids [23]. There have been many reports on the ability of lactic acid bacteria to inhibit the mutagenic potential of mycotoxins. For example Lactobacillus sp has been shown to inhibit mutagenic potential of AFB1, AFB2 and AFG1 in the range of 56.6 - 77.4 %. *Flavobacterium aurantiacumcan* significantly remove AFB1 from a liquid medium and a variety of food products without leaving toxic by-products [1].

Materials and Methods

Collection of Samples

Chemicals

The nine chemicals used in this work were; ammonia solution, 20 % (Merk, Germany), benzoic acid (Merk, Germany), citric acid (Scharlau, Spain), copper sulphate (Sigma-Aldrich, Germany), potassium metabisulfite (Sigma-Aldrich, Germany), propionic acid (Merck, Germany), sodium chloride (Merck), sodium propionate (Alrdich) and urea (Scharlau, Spain).

Herbal Compound/Spices

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

Plants

Nine samples of different plant species, generally available in Pakistan were selected for the study and identified by Botany Department, University of Peshawar, Pakistan. The list of plants tested for antifungal activity, the family to which they belong and parts used is presented (Table II).

Processing of Samples

Chemicals in different weights (0.1 g, 0.2 g and 0.5 g) were added to the freshly prepared growth media of *Aspergillus flavus* (Accession No.1000) and *Aspergillus parasiticus* (Accession No.1002) separately in 250 ml Erlenmeyer flasks containing 100 ml sterile potato dextrose agar (PDA). The concentration obtained was 0.1, 0.2 and 0.5 % for each chemical respectively.

The herbal compounds (ajowain, clove, kalonji) and spices (coriander, cinnamon and turmeric) after sterilization were dried in hot air oven for 1-2 days at 50 ºC, ground to powder form in warring blender and sieved with 1 mm mesh. These samples along with kalonji oil, clove oil and neem oil were added to the growth media of *Aspergillus flavus* and *Aspergillus parasiticus* separately in 250 ml Erlenmeyer flasks containing freshly prepared 100 ml sterile potato dextrose agar (PDA). The concentration obtained was 0.1, 0.2 and 0.5 % for each sample respectively.

The plants were obtained from the local market and immediately kept in refrigerator until starting the experiments. The samples were brought to the laboratory and the healthy parts of the plants were washed thoroughly 2-3 times with running tap water, then with 2% sodium hypochlorite solution and subsequently with sterile distilled water. Different weights of these plants (1.0 g, 2.0 g, and 5.0 g) were dried in hot air oven for 1-2 days at 50 ºC, ground to powder form in warring blender and sieved with 1 mm mesh. These samples were added to the growth media of *Aspergillus flavus* and *Aspergillus parasiticus* separately in 250 ml Erlenmeyer flasks containing freshly prepared 100 ml sterile potato dextrose agar (PDA). The concentration obtained was 0.1, 0.2 and 0.5 % for each sample respectively.

Inhibition of Fungal Growth

Fungal Strains

The pathogenic fungal strains as *Aspergillus flavus* (Accession No.1000) and *Aspergillus parasiticus* (Accession No.1002) were purchased from Institute of Mycology and Plant Pathology; University of the Punjab, Pakistan was used throughout this study.

Preparation of Spore Suspension

A potato dextrose agar (PDA) slant (Oxide, USA) was used for maintaining the cultures of each fungus at 25 ± 2 ºC for 7 days, then spores were removed from the sporulating colonies and poured in sterile distilled water contains Tween 80, 0.1 % (v/v). The suspension of spore was transferred into a sterile bottle and the number of spores was counted by an improved Neubrauer Haemacytometer.

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 × 107 CFU / ml) of *Aspergillus parasiticus* (Accession No.1002) and *Aspergillus flavus* (Accession No.1000) were added, spread, sealed with parafilms and incubated for 7 days at 27–30 ºC 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

The samples which tested the mycelial growth inhibition for *A. flavus* and *A. parasiticus* were further subjected to poisoned food techniques at different concentrations against pathogenic tested fungi. This selection was based on the significant antifungal activity of the screened samples.

Aflatoxin Analysis

All reagents and solvents used were of LC grade supplied by Merck (Darmstadt, Germany). LC grade water was obtained by filtering deionized water through a 0.45µ filter with a Waters Millipore (Milford, MA, USA) system. Solvents and water were degassed for 20 min using ultrasonic bath (Model EIA CP104, Italy). Phosphate buffer saline (PBS) and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO, USA). Standards of aflatoxin B1 (2.02 µg/ ml), aflatoxin B2 (0.520 µg/ ml), aflatoxin G1 (2.01 µg/ ml) and aflatoxin G2 (0.500 µg/ ml) were purchased from Biopure (Tecknopark Tullin, Austria). Standard stock solutions of AFB1, AFB2, AFG1 and AFG2 of concentrations 1 µg/ ml each were prepared by diluting in benzene/ acetonitrile (98:2; v/v). These stock solutions were stored at -4°C in refrigerator, wrapped in aluminum foil due to that aflatoxins gradually breakdown under UV light.

Apparatus

The LC system consisted of Hitachi model L-2000 equipped with two pumps L-2130, auto injector L-2200 and fluorescence detector L-2458 (Macao, Japan). The guard column, Intersil ODS-3, 5 µm (GL sciences Inc. Tokyo, Japan) was placed between the autoinjector and the separative column, Intersil ODS-3 (25cm x 4.6 m I.D., 5 µm, GL science, Tokyo, Japan) column. Photochemical reactor, UVE (LC Tech, Germany) for post column derivatization of aflatoxins was equipped between separative column and fluorescence detector.

Extraction procedure

After autoclaving aflatoxins were analyzed according to the method reported by Stroka, Anklam, Jorissen& Gilbert [26]. Briefly, in 25 g of sample from each flask, 1 g of NaCl added and blended with 40 ml of methanol/water (80:20) and 20 ml of cyclohexane or n-hexane for 3 min. After separation of the two phases, cyclohexane or n-hexane was eliminated. Extracts were filtrated by a Whatman filter paper No. 4.

Immunoaffinity column clean-up

An aliquot of 10 ml was diluted with 60 ml of PBS buffer (pH 7.4). An immune affinity column (IAC AflaTest ®, Vicam, USA) was conditioned with 10 ml of PBS buffer by gentle syringe pressure at a flow rate of 5 ml/min. Then, the mixture of the filtrate diluted extract (70 ml) was applied to the IAC column (1–2 drops per second), followed by a washing with 20 ml of bi distilled water and then dried with air. Aflatoxins were then slowly eluted from the IAC with 2 ml methanol into a glass vial.

LC analysis

The mobile phase consisted of acetonitrile /methanol/ water (8:27:65, v/v/v). The mobile phase was degassed by sonication. The Intersil ODS-3 (25 cm x 4.6 m I.D., 5 µm, GL science, Tokyo, Japan) column was connected as LC column. The column was maintained at 40 ºC with a flow rate of 0.8 ml / min. The aflatoxins were detected at the excitation and emission wavelengths of 365 and 450 nm, respectively. The injection volume was 20 µl.

Statistical Analysis

One way analysis of variance (ANOVA) was applied to data obtained for spore counts and analyses of aflatoxin for each sample separately. The mean values were tested for al significance difference by Ducan's Multiple Range Test (DMRT) at the 5% level [27].

Table I: Common and scientific names of some herbal compounds/spices used to detect their antifungal activities.

Common name	Scientific name	Family	Part used of plant
Ajowain	*Trachyspermum ammi*	Apiaceae	Seed
Clove	*Eugenia caryophyllus*	Myrtaceae	Flower buds
Clove	*Eugenia caryophyllus*	Myrtaceae	Seed oil
Cinnamon	*Cinnamomum burmanii*	Lauraceae	bark
Coriander	*Coriandrum sativum*	Apiaceae	fruit
Kalonji	*Nigela sativa*	Ranunculaceae	Seed
Kalonji	*Nigella sativa*	Ranunculaceae	Oil
Neem	*Azadirachta indica*	Meliaceae	Seed oil
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome

Table II: Common and scientific names of some plants used to detect their antifungal activities.

Common name	Scientific name	Family	Part used of plant
Carrot	*Daucus carota*	Apiaceae	Whole
Garlic	*Allium satium*	Amaryllidaceae	Bulb
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
Lemon grass	*Cymbopogon citratus*	Poaceae	Leaf
Mint	*Mentha longifolia*	Lamiaceae	Leaf
Neem	*Azadirachta indica*	Meliaceae	Leaf
Onion	*Allium cepa*	Amaryllidaceae	Bulb
Sour orange	*Citrus aurantium*	Rutaceae	Peel
Tulsi	*Ocimum sanctum*	Lamiaceae	Leaf

Results and Discussion

Inhibition of Fungal Growth

The inhibition of fungal (*Aspergillus flavus* and *Aspergillus parasiticus*) growth were evaluated. The anti-fungal activity of some chemicals, herbal compounds/spices and plants at different concentrations were evaluated.

Effect of Chemicals on *Aspergillus flavus* and *Aspergillus parasiticus* Growth

Among the chemicals screened, benzoic acid (0.1 – 0.5 %), propionic acid (0.1 – 0.5 %) and copper sulphate (0.2 – 0.5 %) completely inhibited *Aspergillus flavus* growth (Table III). Potassium metabisulphite (0.1 – 0.5 %) and sodium propionate (0.2–0.5 %) exhibited high anti fungal property (64 - 88 %) and (76 – 84 %) respectively. Ammonia (0.2 - 0.5 %), urea (0.2 - 0.5 %) and citric acid (0.2 - 0.5 %) were moderate in inhibiting fungal growth, while ammonia, citric acid and urea below 0.2 % had poor anti-fungal effect.

Among the chemicals screened copper sulphate (0.1 - 0.5 %), benzoic acid (0.2 - 0.5%) and propionic acid (0.2 - 0.5 %) completely inhibited *Aspergillus parasiticus* growth (Table IV). Benzoic acid (0.1 %), potassium metabisulphite (0.1 – 0.5 %), sodium propionate (0.2 –0.5 %) and urea (0.5 %) exhibited high antifungal property as 96 %, 68 - 84 %, 68 - 80 % and 80 % respectively. Ammonia (0.2 - 0.5 %), urea (0.1 - 0.2 %), citric acid (0.5 %) and sodium propionate (0.1 %) were moderate in inhibiting fungal growth, while ammonia (0.1 %), citric acid (0.1 - 0.2 %) and urea (0.1 %) showed poor antifungal property against fungal growth. Except for sodium chloride and urea the antifungal activity of all the remaining chemicals were evaluated. Propionic acid is used to inhibit mould growth. Its disadvantage is that it is a corrosive, which makes it dangerous for handling [28]. The disadvantages of ammoniation are the relatively long period of aeration and its cost which can increase the price of the product by 5–20 % [29].

Effect of Herbal Compounds/Spices on Growth of *Aspergillus flavus* and *Aspergillus parasiticus*

In case of the herbal compounds/spices, clove and clove oil (0.5 %) showed complete inhibition of *Aspergillus flavus* growth (Table V), while clove oil (0.2 %), cinnamon (0.5 %), coriander (0.5 %) and neem oil (0.5 %) had high antifungal activity versus control. Cinnamon (0.2 %), coriander (0.2 %), clove oil (0.1 %), kalonji (0.5 %) and neem oil (0.1 - 0.2%) represented moderate antifungal activity. Ajowain (0.2 - 0.5 %), clove (0.1 - 0.2 %), kalonji (0.1 - 0.2 %), kalonji oil (0.1 - 0.5 %), while cinnamon and coriander (0.1 %) showed poor antifungal activity.

In case of the herbal compounds/spices, clove and clove oil (0.5 %) showed complete inhibition of *Aspergillus parasiticus* growth (Table VI), while clove oil (0.2 %), cinnamon (0.5 %), coriander (0.5 %) and neem oil (0.2 - 0.5 %) showed high antifungal activity. Cinnamon (0.2 %), coriander (0.2 %), clove oil (0.1 %) and kalonji (0.5 %) were moderate in preventing fungal growth. Ajowain (0.2 - 0.5 %), clove (0.1 - 0.2 %), kalonji (0.1 - 0.2 %), kalonji oil (0.1 – 0.5 %), while cinnamon, coriander at below 0.2 % and turmeric at 0.5 % showed low antifungal effect.

It has reported that in wheat and maize grains *Aspergillus flavus* caused production of aflatoxins and essential oils of medicinal plants showed good results by applying basil, cinnamon and thyme [30, 31].The production of AFB1, AFB2, and AFG2 by *A. parasiticus* was completely inhibited at 0.1 µl/ml of clove, with AFG1 production reduced by 66 %. In addition essential oils from 12 different medicinal plants and in particular thyme and cinnamon have been shown to inhibit growth and mycotoxin production in *A. flavus, A. parasiticus, A. ochraceus*, and *F. moniliforme* [31]. The antifungal and antimicrobial activities have reported for of N. sativa Crude extracts [12, 32] and antifungal activities. In Uganda many postharvest techniques have been investigated for the improvement in the quality and shelf life studies of the harvested produce [33].

Effect of Some Plants on Growth of *Aspergillus flavus* and *Aspergillus parasiticus*

Among the plants screened against fungal growth, garlic (0.5 %) and onion (0.5 %) showed complete inhibition of *Aspergillusflavus* growth (Table VII). Ginger (0.5 %), garlic (0.1 - 0.2 %), neem(0.5 %), lemon grass (0.5 %) and onion (0.2 %) had high antifungal activity verses control. Ginger (0.1 - 0.2%), lemon grass (0.2 %), neem(0.1 - 0.2 %) and onion (0.1 %) were moderate in inhibiting fungal growth, while carrot (0.1 - 0.5 %), lemon grass (0.1 %), mint (0.1 - 0.2 %), and sour orange peel (0.2 - 0.5 %) showed poor antifungal effect.

Among the plants screened, garlic (0.5 %) and onion (0.5 %) completely inhibited *Aspergillus parasiticus* growth (Table VIII). Ginger (0.5 %), garlic (0.1 - 0.2 %), neem (0.2 - 0.5 %), lemon grass (0.5 %) and onion (0.2 %) had high antifungal activity. Ginger (0.1 - 0.2 %), lemon grass (0.1 - 0.2 %), mint (0.5 %), neem (0.1 %) and onion (0.1 %) were moderate in inhibiting fungal growth, while carrot (0.1 - 0.5 %), mint (0.1 %), and sour

orange peel (0.5 %) had poor antifungal effect. Tulsi at all concentrations did not show any significant effect against fungal growth. 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 [11, 34].

The production of AFs and the growth of the responsible fungi are dependent upon factors such as temperature, humidity, handling during harvesting and conditions during storage. High moisture and temperature are favorable for the growth of AF producing fungi. Optimum conditions are 16–24% moisture at 20–38 °C. However, it is reported that AF production can also take place at temperatures as low as 7–12 °C [35]. Many plant species like tobacco, tagetes, tephrosia and neem (*Azadirachtaindica*) were found effective for dry cereals and legumes against insect pests. The insect pests such *Acanthoscelides obtectus* and *Sitophilus*, which are common for beans and maize, respectively have been controlled by application of Neem leaves powder and kernel [36].

Table III: Efficacy of some chemicals to inhibit *Aspergillus flavus* growth

Chemicals	Concentration [a]	Spore count [b]	Percent inhibition
Control	–	25 ± 1.65	–
Ammonia (v/v)	0.1 0.2 0.5	19 ± 1.20 9 ± 0.50 5 ± 0.48	24 66 80
Benzoic acid (w/v)	0.1 0.2 0.5	0 0 0	100 100 100
Citric acid (w/v)	0.1 0.2 0.5	18 ± 0.80 15 ± 0.75 11 ± 0.55	28 40 56
Copper sulphate (w/v)	0.1 0.2 0.5	5 ± 0.35 0 0	80 100 100
Potassium metabisulphite (w/v)	0.1 0.2 0.5	9 ± 0.40 7 ± 0.55 3 ± 0.15	64 72 88
Propionic acid (v/v)	0.1 0.2 0.5	0 0 0	100 100 100
Sodium propionate (w/v)	0.1 0.2 0.5	14 ± 0.60 6 ± 0.45 4 ± 0.30	44 76 84
Sodium Chloride (w/v)	0.1 0.2 0.5	25 ± 1.55 24 ± 1.40 23 ± 1.55	0 4 8
Urea (w/v)	0.1 0.2 0.5	15 ± 0.70 9 ± 0.50 6 ± 0.25	40 64 76

a Percentage of potato dextrose agar.
b Colony forming units.

Conclusion

Biological methods were evaluated to decontaminate aflatoxins contaminated agricultural commodities, to prevent human health and the risk of great economic loss. Some materials such as chemicals, herbal compounds/spices and plants showed the inhibitory effect on the growth of *Aspergillus flavus* / *Aspergillus parasiticus*. Among chemicals benzoic acid, propionic acid and copper sulphate were best against fungal growth followed by sodium propionate, potassium metabisulphite, ammonia, urea and citric acid. Among the herbal compounds/spices clove, clove oil were best against fungal growth as compared to cinnamon, coriander, neem oil, kalonji and neem oil. Among the plants garlic and onion showed complete inhibition, while ginger, neem and lemon grass had high antifungal activity. It is concluded that some chemicals, herbal compounds/spices and plants can be utilize as preservatives, which may successfully replace synthetic pesticides and provide an alternative method to protect our staple food from the toxic fungal contamination. The effectiveness of a method depends on the nature of the food, as well as the type of mycotoxin and its concentration level.

Table IV: Efficacy of some chemicals to inhibit *Aspergillus parasiticus* growth

Chemicals	Concentration [a]	Spore count [b]	Percent inhibition
Control	–	25 ± 1.70	–
Ammonia (v/v)	0.1 0.2 0.5	20 ± 1.20 10 ±0.40 6 ±0.25	20 60 76
Benzoic acid (w/v)	0.1 0.2 0.5	1± 0.20 0 0	96 100 100
Citric acid (w/v)	0.1 0.2 0.5	19 ± 0.80 16 ± 0.55 12 ± 0.45	24 36 52
Copper sulphate (w/v)	0.1 0.2 0.5	0 0 0	100 100 100
Potassium metabisulphite (w/v)	0.1 0.2 0.5	8 ± 0.35 6 ± 0.32 4 ± 0.15	68 76 84
Propionic acid (v/v)	0.1 0.2 0.5	2 ± 0.10 0 0	92 100 100
Sodium Chloride (w/v)	0.1 0.2 0.5	25 ± 1.40 27 ± 1.25 27 ± 0.88	0 8 8
Sodium propionate (w/v)	0.1 0.2 0.5	16 ± 0.75 17 ± 0.60 16 ± 0.54	44 68 80
Urea (w/v)	0.1 0.2 0.5	15 ± 0.85 10 ± 0.70 5 ± 0.50	40 60 80

a Percentage of potato dextrose agar.
b Colony forming units.

Table V: Efficacy of some herbal compounds/spices to inhibit *Aspergillus flavus* growth

Herbal Compounds/Spices	Concentration [a]	Spore count [b]	Percent inhibition
Control	–	25 ± 1.60	–
Ajowain (w/v)	0.1 0.2 0.5	25 ± 1.45 21 ± 1.60 16 ± 0.58	0 16 36
Clove (w/v)	0.1 0.2 0.5	18 ± 1.20 16 ± 0.75 0	28 36 100
Clove oil (v/v)	0.1 0.2 0.5	15 ± 0.66 9 ± 0.35 0	40 64 100
Cinnamon (w/v)	0.1 0.2 0.5	16 ± 1.10 12 ± 0.85 3 ± 0.12	36 52 88
Coriander (w/v)	0.1 0.2 0.5	17 ± 0.45 15 ± 0.50 7 ± 0.60	32 40 72
Kalonji (w/v)	0.1 0.2 0.5	19 ± 1.45 18 ± 1.20 12 ± 0.75	24 28 52
Kalonji oil (v/v)	0.1 0.2 0.5	21 ± 1.30 20 ± 1.10 20 ± 1.55	16 20 20
Neem oil (v/v)	0.1 0.2 0.5	14 ± 0.76 11 ± 0.45 2± 0.25	44 56 92
Turmeric (w/v)	0.1 0.2 0.5	25 ± 1.60 23 ± 1.35 22± 0.95	0 8 12

a Percentage of potato dextrose agar.
b Colony forming units.

Table VI: Efficacy of some herbal compounds/spices to inhibit *Aspergillus parasiticus* growth

Herbal Compounds/ Spices	Concentration [a]	Spore count [b]	Percent inhibition
Control	–	25 ± 2.05	–
Ajowain (w/v)	0.1 0.2 0.5	25 ± 1.45 22 ± 1.60 16 ± 1.80	0 12 36
Clove (w/v)	0.1 0.2 0.5	17 ± 1.20 15 ± 0.65 0	32 40 100
Clove oil (v/v)	0.1 0.2 0.5	14 ± 0.30 10 ± 0.25 0	44 60 100
Cinnamon (w/v)	0.1 0.2 0.5	15 ± 1.20 11 ± 0.45 3 ± 0.15	40 56 88
Coriander (w/v)	0.1 0.2 0.5	16 ± 0.70 14 ± 0.65 6 ± 0.50	36 44 76
Kalonji (w/v)	0.1 0.2 0.5	19 ± 1.25 18 ± 1.16 12 ± 0.54	24 28 52
Kalonji oil (v/v)	0.1 0.2 0.5	21 ± 1.55 21 ± 1.40 19 ± 1.35	16 16 24
Neem oil (v/v)	0.1 0.2 0.5	13 ± 0.75 10 ± 0.40 2 ± 0.30	48 60 92
Turmeric (w/v)	0.1 0.2 0.5	25 ± 1.70 22 ± 1.45 16 ± 0.85	0 12 24

a Percentage of potato dextrose agar.
b Colony forming units.

Table VII: Efficacy of some plants to inhibit *Aspergillus flavus* growth

Plants	Concentration [a]	Spore count [b]	Percent inhibition
Control	–	25 ± 1.80	–
Carrot (w/v)	0.1 0.2 0.5	19 ± 2.30 16 ± 1.48 15 ± 1.25	24 36 40
Garlic (w/v)	0.1 0.2 0.5	7 ± 0.45 5 ± 0.30 0	72 80 100
Ginger (w/v)	0.1 0.2 0.5	11 ± 0.50 9 ± 0.45 4 ± 0.15	56 64 84
Lemon grass (w/v)	0.1 0.2 0.5	14 ± 0.56 11 ± 0.45 5 ± 0.20	44 56 80
Mint (w/v)	0.1 0.2 0.5	15 ± 0.60 15 ± 0.53 10 ± 0.25	40 40 60
Neem (w/v)	0.1 0.2 0.5	12 ± 0.80 9 ± 0.55 3 ± 0.05	52 64 88
Onion (w/v)	0.1 0.2 0.5	10 ± 0.35 4 ± 0.20 0	60 84 100
Sour orange peel (w/v)	0.1 0.2 0.5	25 ± 2.15 20 ± 1.75 18 ± 0.95	0 16 28
Tulsi (w/v)	0.1 0.2 0.5	25 ± 1.90 23 ± 1.65 22 ± 1.40	0 8 12

a Percentage of potato dextrose agar;
b Colony forming units.

Table VIII: Efficacy of some plants to inhibit *Aspergillus parasiticus* growth

Plant	Concentration [a]	Spore count [b]	Percent inhibition
Control	–	25 ± 1.65	–
Carrot (w/v)	0.1 0.2 0.5	20 ± 1.56 17 ± 1.28 15 ± 1.20	20 32 40
Garlic (w/v)	0.1 0.2 0.5	6 ± 0.54 4 ± 0.35 0	76 84 100
Ginger (w/v)	0.1 0.2 0.5	12 ± 0.60 10 ± 0.55 4 ± 0.36	52 60 84
Lemon grass (w/v)	0.1 0.2 0.5	12 ± 1.30 11 ± 0.85 6 ± 0.32	52 56 76
Mint (w/v)	0.1 0.2 0.5	14 ± 0.70 12 ± 0.65 9 ± 0.54	44 52 64
Neem (w/v)	0.1 0.2 0.5	13 ± 1.10 7 ± 0.68 2 ± 0.25	48 72 92
Onion (w/v)	0.1 0.2 0.5	11 ± 0.85 3 ± 0.30 0	56 88 100
Sour orange peel (w/v)	0.1 0.2 0.5	25 ± 2.15 22 ± 1.75 18 ± 1.36	0 12 28
Tulsi (w/v)	0.1 0.2 0.5	25 ± 1.56 23 ± 1.40 23 ± 1.20	0 8 8

a Percentage of potato dextrose agar;
b Colony forming units.

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