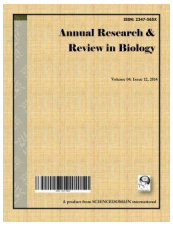

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Aflatoxigenic Fungi Associated with Some Medicinal Plants

Fatma F. Migahed[1], Manar. M. Abdel-Gwad[1] and Sherif R. Mohamed[2*]

[1]Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt.
[2]Department of Food Toxicology and Contaminants, National Research Centre, Dokki, Giza, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MMAG, FFM and SRM designed the study. Author MMAG performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FFM and SRM managed the analyses of the study. Author SRM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: A total of one hundred and forty four samples belonging to 48 kinds of dried medicinal plants collected from different markets in Mansoura city, Dakahlia governorate were examined for the natural occurrence of molds and aflatoxins.
Methods: All the samples were analyzed for aflatoxin contamination using TLC and HPLC techniques.
Results: There is a high occurrence of the various fungal population of the analyzed samples and their counts ranged between 5-100 colonies/g. Thirty six species and 1 variety belonging to 11 genera were isolated using standard potato dextrose agar plate method. The most heavily contaminated samples were observed in anise and linseeds in order of magnitude of 95 and 100 colony/g, while sumac, clove and cinnamon revealed no fungal contamination indicating their antifungal properties. The genus *Aspergillus* (91.7%) was encountered as the most frequent fungal genus recorded, followed by *Penicillium* (68.8%). The most frequent fungal species were

**Corresponding author: E-mail: sheriframzy4@gmail.com;*

Aspergillus niger, *Penicillium chrysogenum*, *Aspergillus flavus* var *columnaris* and *Aspergillus flavus* comprising 75%, 56.3%, 41.7% and 33.3% in their frequency of occurrence. Out of 151 isolates of *Aspergillus flavus* group isolated from various samples, 67 isolates (44.4%) were found to be toxigenic and their potential to produce aflatoxins was in the range of 0.1 to 818.2 ng/ml of culture filtrate. Of 30 medicinal plant samples screened, 19 samples (63.3%) were found contaminated with aflatoxins in the range of 1.5 to 724.6 ng/g.

Conclusion: These results revealed that the selected medicinal plants are heavily contaminated with fungal strains and more strict measures must be taken to prevent mold contamination and hence aflatoxins production in medicinal plants.

Keywords: Medicinal plants; Mycoflora; Fungi; Aspergillus flavus; Aflatoxins.

1. INTRODUCTION

The safety of medicinal plants has been increasingly concerned worldwide as the number of people using such plants as alternative therapy or self-medication increases [1]. They are used for therapeutical and medicinal purposes due to the active ingredients. However, like many other agricultural products medicinal plants and spices may be contaminated by a wide range of fungi [2,3], such as *Aspergillus flavus* section and *Alternaria alternate* section, as well as *Penicillia* and *Scopulariopsisetc* [4,5], from soil or plants during the procedure of growth, harvest, process, storage under favorable conditions of temperature and humidity and transportation [5,6]. Therefore, fungal contamination is very critical for the economic, food safety and human health perspectives [5]. Fungal contamination of stored medicinal plants is not only linked to discoloration, quality deterioration, reduction in commercial value as well as in therapeutic potential, but also causes the hazard of mycotoxins production [7-9]. Mycotoxins are toxic secondary metabolites produced by some species of fungal genera such as *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* [10]. Among all mycotoxins, aflatoxins are naturally occurring secondary metabolites from some species of *Aspergillus* and they are carcinogenic, mutagenic, teratogenic and immunosuppressive to most animal species and humans [11,12]. *Aspergillus flavus* and *Aspergillus parasiticus* are the main producers of AFs: aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) [13].

The main purpose of this investigation was to evaluate the mycoflora associated with some medicinal plants and their potential to produce aflatoxins in order to achieve some notes on the presence of a health hazard to individuals consuming these plants and to use this information in disease prevention or control.

2. MATERIALS AND METHODS

2.1 Collection of Samples

One hundred and forty four samples of 48 different types of dried and powdered medicinal plants were collected randomly from different herbal markets in Mansoura city, Dakahlia governorate, Egypt during the period from December, 2013 to February, 2015.

2.2 Isolation and Identification of Fungi

The total number of fungal colonies was determined by agar plate method [14]. About 0.2 gram of each sample was transferred with sterile forceps into a Petri dish containing sterilized potato dextrose agar medium (PDA) (200 g potato, 20 g dextrose, 20 g agar and 1 L distilled water) containing 0.5 mg chloramphenicol/L medium to suppress bacterial growth. Three replicates were made and the plates were incubated at 28±2°C for 5-7 days. After incubation, the growing fungal colonies were isolated and the purity of each isolate was examined by streaking the isolate over Czapeck-Dox agar medium (30 g sucrose, 3 g sodium nitrate, 0.5 g potassium chloride, 0.5 g magnesium sulfate, 0.01 g ferrous sulfate, 1 g dibasic potassium phosphate, 20 g agar and 1 L distilled water) then it was transferred to potato dextrose agar medium (PDA) slants which kept at 4°C until identification. The number of fungal colonies was counted and calculate/g of each sample. The relative density (RD) and frequency of occurrence (FR) of species were calculated as follows [15]:

$$\text{RD (\%)} = \frac{Number\ of\ isolates\ of\ a\ species\ or\ genus}{Total\ number\ of\ fungal\ isolates} \times 100$$

$$\text{FR (\%)} = \frac{Number\ of\ samples\ with\ a\ species\ or\ genus}{Total\ number\ of\ samples} \times 100$$

The identification of fungi was done based on morphological and microscopic characteristics,

sporulation and colony color with the help of universally accepted keys for identification [16-25].

2.2.1 Aflatoxins analysis

2.2.1.1Extraction of aflatoxins from the culture media

The extraction was performed according to the procedure offered by Kumar, A., et al. [26]. with some modifications. The cultures were filtered and mycelial mats were collected. Aflatoxins were extracted from culture filtrates with chloroform. A known volume of filtrate (25 ml) was added to 10 ml chloroform and was shaken for half hour. The chloroform contained aflatoxins were separated by separating funnel which was allowed to stand for some time until the two layers appeared. The upper aqueous layer was re-extracted many times with chloroform for complete separation. The lower chloroform layer was filtered over anhydrous sodium sulfate in 250 ml beaker, evaporated in a water bath (70-80°C) near dryness and the residue was washed twice with chloroform (1-2ml) into a glass vial which evaporated till dryness (dry film). The dried extract was kept in the refrigerator at -5°C until analysis.

2.2.2 Extraction of aflatoxins from medicinal plant samples

Extraction of aflatoxins from medicinal plant samples were carried out according to the best foods (BF) method [27]. Twenty-five grams of each homogenized sample was weighed and transferred to 500 ml glass-stoppered Erlenmeyer flask and 125 ml methanol-water (55+45), 100 ml hexane and 2 gm sodium chloride were added. Shaking vigorously for 30 min on an orbital shaker then the mixture was filtered through Whatman No.1 filter paper and the filtrate was allowed to stand and separation occurred within 30 minutes. The 25 ml of lower aqueous methanol phase was taken in a separating funnel and 10 ml chloroform was added then shaken for 30-60 seconds. The process was repeated several times with chloroform for complete separation. The separating funnel was allowed to stand for some time until the two layers formed, then the lower chloroform layer was drained over anhydrous sodium sulfate into a 250 ml beaker. Collected in a beaker and evaporated combined chloroform extract in a water bath (50-60°C) near dryness and the residue was washed twice with chloroform (1-2 ml) into a glass vial which was evaporated till dryness (dry film).

2.2.3 Thin layer chromatography (TLC) analysis

Aflatoxins were detected according to the method of Calvo, AM., et al. [28]. Extracts of fungal cultures grown on broth media and extracts from selected substrates were screened for aflatoxins production. Volume of 20 µl of each extract and aflatoxins standards were spotted on thin layer chromatography silica gel plate and was then transferred to a jar containing the following solvent system, toluene: ethyl acetate: formic acid (6:3:1), which was found to be the most favorable for resolution. The solvent system was allowed to rise until it almost reached the top of the plate, which will give the maximum separation of the extract components. The plate was then observed under long wave ultraviolet light for the presence of aflatoxins by their distinctive fluorescence properties. The blue fluorescence corresponding to aflatoxins B1 and B2 while the green fluorescence corresponding to G1 and G2. The intensity of fluorescence and colors of the extracts were compared with various concentrations of aflatoxins standards. The sample extracts which were positive for the presence of aflatoxins were taken for HPLC analysis.

2.2.4 High performance liquid chromatography (HPLC) analysis

According to AOAC [29]. The HPLC system used was a water 600-pump system equipped with model 474-flourescence detector (water) set at 360 nm for excitation and 440 nm for emission wavelength. Water Nova-pack C18 column (150×3.9) was used for aflatoxins separation. The mobile phase (water: Acetonitrile: Methanol, 65: 5: 30) was isocratically flow at rate of 1.0 ml/min. Data were collected and integrated with a waters Millennium 32 chromatography Manager software program.

3. RESULTS AND DISCUSSION

3.1 Mycoflora Associated with Medicinal Plants

The fungal populations isolated from the medicinal plant samples are shown in Table (2). In all cases, a total of thirty six species and 1 variety belonging to twelve genera were isolated and identified as: *Acremoniella, Alternaria, Aspergillus, Botryotrichum, Fusarium, Myrothecium, Penicillium, Rhizopus,*

Scopulariopsis, Torula, Trichoderma, Ulocladium and sterile mycelium (dark colour). Similar observations were reported by [30-34] and several others. [35] reported that the major field fungi genera are: *Alternaria*, *Helminthosporium*, *Fusarium*, and *Cladosporium* and the storage molds are principally species of *Aspergillus* and *Penicillium*.

Table 1. Types of medicinal plants used in the study

No	Common name	Scientific name	Part of plant used
1	Ammi	*Ammi majus*	Leaves & flowers
2	Anise	*Pimpniella anisum*	Seeds
3	Bay leaf	*Laurus nobilis*	Leaves
4	Black tea	*Thea sinensis*	Leaves
5	Black pepper	*Piper nigrum*	Dried fruits
6	Cacao	*Theobroma cacao*	Seeds
7	Caraway	*Carum carvi*	Seeds
8	Cardamom	*Elleteria cardamomum*	Seeds
9	Carob	*Ceratonia siliqua*	Dry fruits
10	Castor beans	*Ricinus communis*	Seeds
11	Chamomile	*Metricaria chamomile*	Leaves & stems
12	Cinnamon	*Cinnamomum zeylanicum*	Stem bark
13	Clove	*Eugenia Caryophyllus*	Flower buds
14	Coriander	*Coriandrum sativum*	Seeds
15	Cress seeds	*Lepidium sativum*	Seeds
16	Cumin	*Cuminum cyminum*	Seeds
17	Dill	*Anethum graveolens*	Seeds
18	Eucalyptus	*Eucalyptus globulus*	Leaves
19	Fennel	*Foeniculum vulgare*	Seeds
20	Fenugreek	*Trigonella foenum-graecum*	Seeds
21	Garlic	*Allium sativum*	Bulb
22	Ginger	*Zingiber officinale*	Dry rhizomes
23	Green te	*Camellia sinensis*	Leaves
24	Guava leaf	*Psidium guajava*	Leaves
25	Karkade	*Hibiscus sabdariffa*	Flowers
26	Linseed	*Linum usitatissimum*	Seeds
27	Liquorice	*Glycyrrhiza glabra*	Rhizomes
28	Majoram	*Origanum majorana*	Leaves
29	Moghat	*Glossostemon bruguieri*	Roots
30	Mustard	*Brassica alba*	Seeds
31	Nigella	*Nigella sativa*	Seeds
32	Nutmeg	*Myristica fragrans*	Peeled seeds
33	Parsley	*Petroselinum sativum*	Seeds
34	Pepper mint	*Mentha spicata*	Leaves
35	Radish	*Raphanus sativus*	Seeds
36	Red pepper	*Capsicum frutescens*	Fruits
37	Rocket	*Eruca sativa*	Seeds
38	Rosemary	*Rosmarinus officinalis*	Leaves
39	Safforn	*Crocus sativus*	Flowers
40	Sagebrush	*Salvia officinalis*	Leaves
41	Santonica	*Artemisia absintham*	Flowers
42	Senna	*Cassia italic*	Leaves
43	Sumac	*Rhus coriaria*	Dried fruits
44	Sweet basil	*Ocimum basilicum*	Leaves
45	Tamarind	*Tamarindus indica*	Seeds
46	Thyme	*Thymus vulgaris*	Leaves
47	Tilia	*Tilia ulmifolia*	Leaves
48	Turmeric	*Curcuma longa*	Rhizomes

Table 2. Total count (colonies/g) of fungi isolated from different medicinal plant samples by agar plate method at 28±2°C

Genera & species \ Medicinal plants	Anise	Bay leaf	Black tea	Black pepper	Cacao	Caraway	Cardamom	Carob	Castor beans	Chamomile	Cinnamon	Clove	Coriander	Cress seeds	Cumin	Dill	Eucalyptus	Fennel	Fenugreek	Garlic	Ginger	Green tea	Guava leaves	Karkade	Khella	Linseeds	Liquorice	Majoram	Moghat	Mustard	Nigella	Nutmeg	Parsley	peppermint	Radish	Red pepper	Rocket	Rosemary	Saffron	Sagebrush	Santonica	Senna	Sumac	Sweet basil	Tamarind	Thyme	Tilia	Turmeric	% TC
Acremoniella spp	–	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.48
Alternaria	5	–	–	–	–	–	–	–	2	10	–	–	–	20	5	–	–	–	8	–	–	–	–	–	–	–	–	–	15	5	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	3.5
A. alternata	5	–	–	–	–	–	–	–	2	–	–	–	–	20	5	–	–	–	8	–	–	–	–	–	–	–	–	–	15	5	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	3.1
A. cineraria	–	–	–	–	–	–	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.48
Aspergillus	68	28	44	55	47	20	37	25	18	18	–	–	8	18	15	15	13	51	31	8	30	10	–	25	15	80	17	35	43	28	45	12	51	30	26	5	10	15	16	55	70	24	–	50	16	55	33	15	64.3
A. avenaceus	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.34
A. clavato-flavus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05
A. flavus	–	–	–	7	–	–	20	–	–	–	–	–	–	–	2	–	–	–	5	–	–	–	–	5	–	30	2	–	18	–	10	–	–	5	5	–	5	–	3	15	12	–	–	–	–	5	3	–	7.1
A .flavus var columnaris	10	–	–	–	5	–	–	20	8	–	–	–	–	5	–	–	–	5	5	–	20	–	–	–	5	20	–	5	5	8	13	–	3	–	5	5	–	5	5	–	–	25	12	–	–	–	–	–	–	9.1
A. fumigatus	8	5	–	–	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	5	–	–	–	–	5	–	–	–	–	1	–	–	–	–	–	1	–	5	–	–	2.2
A. glaucaus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24
A. nidulans	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24

Table 2 continue

Genera & species \ Medicinal plants	Anise	Bay leaf	Black tea	Black pepper	Cacao	Caraway	Cardamom	Carob	Castor beans	Chamomile	Cinnamon	Clove	Coriander	Cress seeds	Cumin	Dill	Eucalyptus	Fennel	Fenugreek	Garlic	Ginger	Green tea	Guava leaves	Karkade	Khella	Linseeds	Liquorice	Majoram	Moghat	Mustard	Nigella	Nutmeg	Parsley	peppermint	Radish	Red pepper	Rocket	Rosemary	Saffron	Sagebrush	Santonica	Senna	Sumac	Sweet basil	Tamarind	Thyme	Tilia	Turmeric	% TC
A. niger	30	10	27	15	11	15	10	5	–	8	–	–	3	5	3	10	5	25	8	–	5	10	–	20	–	–	–	7	30	15	5	17	–	14	10	16	5	–	10	8	26	25	–	–	22	10	25	7	–	23.1
A. ochraecous	5	6	10	20	–	–	–	–	–	–	–	–	–	–	3	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	22	–	–	–	–	–	–	3	–	–	25	3	5	10	5	5.9
A. ornatus	5	–	–	5	–	–	–	–	5	–	–	–	–	8	–	–	3	13	13	–	–	–	–	–	–	30	–	–	–	10	–	6	10	5	–	–	–	–	–	–	–	–	–	–	–	–	–	5.5
A. oryzae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	2	–	5	–	–	–	–	–	–	–	–	5	–	–	–	–	–	2	–	–	5	1.2
A. parasiticus	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	5	–	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	1.3
A. sulphureus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24
A. tamarii	10	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	10	–	3	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.3
A. terreus	–	7	–	–	8	5	–	–	5	10	–	–	–	–	5	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	–	15	5	–	–	–	–	–	–	–	–	2.8
A. versicolor	–	–	–	8	–	–	7	–	–	–	–	–	–	–	5	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	5	–	–	10	–	–	–	–	3	7	–	–	–	3	–	15	13	–	3.8
Botryotrichum piluliferum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	8	–	–	–	3	5	–	–	–	0.77
Fusarium	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	1.6

Table 2 continue

Genera & species \ Medicinal plants	Anise	Bay leaf	Black tea	Black pepper	Cacao	Caraway	Cardamom	Carob	Castor beans	Chamomile	Cinnamon	Clove	Coriander	Cress seeds	Cumin	Dill	Eucalyptus	Fennel	Fenugreek	Garlic	Ginger	Green tea	Guava leaves	Karkade	Khella	Linseeds	Liquorice	Majoram	Moghat	Mustard	Nigella	Nutmeg	Parsley	peppermint	Radish	Red pepper	Rocket	Rosemary	Saffron	Sagebrush	Santonica	Senna	Sumac	Sweet basil	Tamarind	Thyme	Tilia	Turmeric	% TC
F. moniliforme	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	–	–	–	–	–	5	–	–	0.73
F. oxysporum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	0.15
F. solani	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.73
Myrothecium spp	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24
Penicillium	10	15	5	5	–	20	5	5	6	40	–	–	–	20	10	2	5	8	25	–	10	–	5	–	14	15	33	42	10	2	10	15	12	5	–	–	10	–	5	–	10	–	5	–	15	–	25	20.5
P. chrysogenum	10	10	5	–	–	20	5	–	6	15	–	–	–	20	10	–	–	8	25	–	10	–	5	–	14	15	33	20	10	–	10	15	12	5	–	–	10	–	5	–	10	–	–	5	15	–	–	15.9
P. citrinum	–	–	–	–	–	–	–	5	–	5	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	22	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	25	3.1
P. purpurogenum	–	5	–	5	–	–	–	–	–	10	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.1
P. raistrickii	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24
P. waksmanii	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24
Rhizopus	5	3	–	–	–	–	6	–	–	–	–	–	–	–	6	3	3	–	–	10	–	6	–	–	7	5	–	–	–	5	–	–	–	5	2	7	–	–	–	–	5	–	–	3	–	9	–	4.4

Table 2 continue

Genera & species \ Medicinal plants	Anise	Bay leaf	Black tea	Black pepper	Cacao	Caraway	Cardamom	Carob	Castor beans	Chamomile	Cinnamon	Clove	Coriander	Cress seeds	Cumin	Dill	Eucalyptus	Fennel	Fenugreek	Garlic	Ginger	Green tea	Guava leaves	Karkade	Khella	Linseeds	Liquorice	Majoram	Moghat	Mustard	Nigella	Nutmeg	Parsley	peppermint	Radish	Red pepper	Rocket	Rosemary	Saffron	Sagebrush	Santonica	Senna	Sumac	Sweet basil	Tamarind	Thyme	Tilia	Turmeric	% TC
R. nigricans	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	–	–	–	–	7	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.87
R. stolonifer	5	3	–	–	–	–	6	–	–	–	–	–	–	–	–	3	3	–	–	10	–	6	–	–	–	5	–	–	–	–	–	–	–	5	2	7	–	–	–	–	–	–	–	3	–	9	–	–	3.5
Scopulariopsis	–	–	–	–	–	–	–	–	–	5	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	20	–	–	–	–	–	–	–	–	–	11	–	–	–	–	–	–	–	–	2.1
S. brevicaulis	–	–	–	–	–	–	–	–	–	5	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.6
S. brumptii	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11	–	–	–	–	–	–	–	–	0.53
Torula graminis	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24
Trichoderma harzianum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	5	–	–	20.5
Ulocladium alternaria	7	–	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	0.97
Sterile mycelium	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24
Total counts	95	46	49	60	57	40	48	30	26	81	–	–	13	66	36	20	24	67	64	18	40	16	5	25	36	100	50	77	83	70	55	27	63	35	31	17	30	15	21	74	80	29	–	65	22	75	47	40	2068
Number of genera	5	3	2	2	2	2	3	2	3	5	–	–	2	4	4	3	4	3	3	2	2	2	1	1	3	3	2	2	4	6	2	2	2	2	2	3	4	1	2	3	2	2	–	4	3	3	3	2	12
Number of species	10	7	4	4	6	3	5	3	5	10	–	–	3	6	9	4	6	7	6	3	4	2	1	2	4	4	5	6	4	7	9+1	5	3	6	5	4	3	5	2	4	8	5	3	0	6	6	7	6	36+1 var.

Furthermore, the results obtained in Table (2) detected also that, the highest contaminated samples were those of linseed (TC=100 colonies/g), anise (TC=95 colonies/g), moghat (TC=83 colonies/g), chamomile (TC=81 colonies/g), santonica (TC=80 colonies/g), majoram (TC=77colonies/g), thyme(TC=75 colonies/g), sage (TC=71 colonies/g) and mustard (TC=70 colonies/g). In this respect, [30] reported that anise was highly contaminated with fungi. [36] demonstrated that samples of linseed were highly contaminated with fungi having a total of 10 fungal genera represented by 18 species including *Aspergillus*, *Penicillium*, *Alternaria*, *Curvularia*, *Fusarium*, *Tricoderma* and *Mucor*. Also, our results were in agreement with those previously reported by [37-39]. The least contaminated substrates were coriander and guava leaves (TC= 13 and 5 colonies/g respectively); this was in accordance with results reported by [4,40,41]. In contrast, samples of cinnamon, clove and sumac showed no fungal contamination indicating their antifungal properties. The current observations were supported by [34,40,42,43].

Of all genera detected throughout this study (Table 3), *Aspergillus* was the most frequent and prevalent genus encountering 91.7% of all medicinal plant samples examined yielding 64.3% of the total count of fungi as indicated by [38,43-46] and many others. Sixteen species of *Aspergillus* namely: *A. avenaceus*, *A. clavato-flavus*, *A.flavus, A. flavus var columnaris*, *A. fumigatus*, *A. glaucaus*, *A. nidulans, A. niger*, *A. ochraecous*, *A. ornatus*, *A. oryzae*, *A. parasiticus*, *A. sulphureus*, *A. tamari*, *A. terreus* and *A. versicolor* were identified. *Aspergillus niger*, *Aspergillus flavus var columnaris* and *Aspergillus flavus* were the most frequently encountered and widely distributed species detected in almost all samples comprising 75%, 41.7% and 33.3% of their frequency of occurrence yielding 23.1%, 9.1% and 7.1% of the total count of fungi respectively. In this respect, [33,47] stated that the most prevalent fungi isolated from some medicinal plants samples were *A. flavus*, *A. niger* and *A. parasiticus*. Also, our results are in well aggrement with [34,46,48]. All of the other species were isolated in low or rare frequency of occurrence except *Aspergillus ochraecous* and *A. ornatus* which were detected in moderate frequency of occurrence.

As illustrated in Table (3), *Penicillium* was the second frequent genus comprising 68.8% of all medicinal plant samples analyzed constituting 20.5% of its density of occurrence. It was represented by five species namely: *Penicillium chrysogenum*, *P. citrinum*, *P. purpurogenum*, *P. raistrickii* and *P. waksmanii* of which *P. chrysogenum* was the most common species recorded encountering 56.3% amongst all samples and 15.9% of the total count of fungi of all samples followed by *P. citrinum* representing 12.5% of its frequency of occurrence and 3.1% of the total count of fungi respectively. All of the other species were detected in rare frequency of occurrence. In this respect, [36] examined fungal contamination of 50 samples of linseeds on Czapek Dox agar medium at 28°C and found that *Penicillium citrinum* was the second abundant species comprising 24% of all samples analyzed. [44] recorded that *Penicillium* was isolated in high frequency of occurrence from 50 samples of different kinds of spices accounting 80% of the samples and was represented by 5 species of which *P.chrysogenum* and *P. corylophilum* were the most prevalent. Almost similar species were previously recovered from different types of medicinal plants but with different densities and frequencies [4,34,47,49].

On the other hand, *Rhizopus* was ranked third after *Aspergillus* and *Peniciliium* and recorded in moderate frequency of occurrence comprising 35.4% of all samples studied and 4.4% of the total count of fungi. Two species namely: *Rhizopus nigricans* and *Rhizopus stolonifer* were identified of which *R. stolonifer* was the most frequent comprising 29.2% of all samples analyzed. Similar observations were reported by [4,40,50-52].

The genus *Alternaria* was detected in low frequency of occurrence representing 18.8% of all samples and 3.5% of the total count of fungi. It was represented by 2 species of which *Alternaria alternate* was the most frequent recorded in 16.7% of all samples studied as indicated by [49] who stated that *A. alternata* ranked third and was represented in 40% of the samples constituting 2.8% of the total fungi isolated. Furthermore, *Scopulariopsis* and *Fusarium* were recorded in 8.3% of all samples *Scopulariopsis* was represented by two species while *Fusarium* was represented by three species and all of their species were detected in rare frequency of occurrence. These results came in agreement with [33]. The remaining genera were represented by one species only and isolated from one or two substrates with rare frequency of occurrence as shown in Table (3).

3.2 Aflatoxins Production by Fungal Strains in the Culture Media

It is clear from Table (4) that 67 isolates (44.4%) out of 151 isolates of *A. flavus* group isolated from various samples were found to be toxigenic and their potentiality to produce aflatoxins was in the range of 0.1 to 818.2 ng/ml of culture filtrate. In this respect, [31] found that 21 isolates out of 50 of *A. flavus* isolated from medicinal plant samples were toxigenic and the amount of aflatoxin B_1 they produced ranged from 0.09 to 0.65 µg/ml of culture filtrate. Also, [53] revealed that about 50% of the *A. flavus* isolates were found to produce aflatoxin-B_1 in the range of (0.02 to 2.66 µg/ml of culture filtrate).

Table 3. Total counts, % frequency of occurrence, number of cases of isolation and occurrence remarks of fungal genera and species

Fungal genera and species	Total count	% frequency of occurrence	NCI and OR
Acremoniella spp	10	2.1	1R
Alternaria	73	18.8	9L
A. alternata	63	16.7	8L
A. cineraria	10	2.1	1R
Aspergillus	1327	91.7	44H
A. avenaceus	7	2.1	1R
A. clavato-flavus	1	2.1	1R
A. flavus	124	33.3	16M
A. flavus var columnaris	209	41.7	20M
A. fumigatus	46	16.7	8L
A. glaucaus	5	2.1	1R
A. nidulans	5	2.1	1R
A. niger	477	75	36H
A. ochraecous	122	27.1	13M
A. ornatus	113	25	12M
A. oryzae	24	12.5	6L
A. parasiticus	26	10.4	5R
A. sulphureus	5	2.1	1R
A. tamarii	26	10.4	5R
A. terreus	58	16.7	8L
A. versicolor	79	22.9	11L
Botryotrichum piluliferum	16	4.2	2R
Fusarium	33	8.3	4R
F. moniliforme	15	4.2	2R
F. oxysporum	3	2.1	1R
F. solani	15	2.1	1R
Myrothecium spp	5	2.1	1R
Penicillium	424	68.8	33H
P. chrysogenum	328	56.3	27H
P. citrinum	64	12.5	6L
P. purpurogenum	22	8.3	4R
P. raistrickii	5	2.1	1R
P. waksmanii	5	2.1	1R
Rhizopus	90	35.4	17M
R. nigricans	18	6.25	3R
R. stolonifer	72	29.2	14M
Scopulariopsis	44	8.3	4R
S. brevicaulis	33	6.25	3R
S. brumptii	11	2.1	1R
Torula graminis	5	2.1	1R
Trichoderma harzianum	13	6.25	3R
Ulocladium alternaria	20	6.25	3R
Sterile mycelium	5	2.1	1R

NCI - Number of cases of isolation

OR - Occurrence remarks: H = High occurrence; more than 24 cases; M = Moderate occurrence; between 12-24 cases; L = Low occurrence; between 6-11 cases; R = Rare occurrence; less than 6 cases

Table 4. Aflatoxins production (ng/ml) on Yeast extract-sucrose broth medium by some fungal isolates at 28±2°C

Medicinal plants	Fungal isolates	Isolate No.	Aflatoxins concentrations (ng/ml)				
			B1	B2	G1	G2	Total
Ammi	*Aspergillus flavus var columnaris*	1	54.6	18	-	-	72.6
	A. flavus var columnaris	2	-	-	-	-	-
Anise	*A. flavus var columnaris*	3	-	-	-	-	-
	A. flavus var columnaris	4	1.8	-	7.3	-	9.1
	A. flavus var columnaris	5	-	-	4.9	-	4.9
	A. flavus var columnaris	6	-	-	3.5	-	3.5
	A. flavus var columnaris	7	1.7	-	6.2	-	7.9
	A. flavus var columnaris	8	-	-	-	-	-
	A. flavus var columnaris	9	-	-	-	-	-
	A. flavus var columnaris	10	-	-	-	-	-
	A. flavus var columnaris	11	-	-	15.3	-	15.3
	A. flavus var columnaris	12	-	-	-	-	-
	A. flavus var columnaris	13	-	-	-	-	-
	A. flavus var columnaris	14	-	-	-	-	-
	A. flavus var columnaris	15	-	-	-	-	-
	A. flavus var columnaris	16	-	-	-	-	-
	A. flavus var columnaris	17	-	-	10	1.8	11.8
	A. flavus var columnaris	18	-	-	-	-	-
	A. flavus var columnaris	19	-	-	-	-	-
Black pepper	*A. flavus*	20	768.3	-	22.6	-	790.9
	A. flavus	21	-	-	-	-	-
	A. flavus	22	133	-	18.2	-	151.2
	A. flavus	23	-	-	-	-	-
Cacao	*A. flavus var columnaris*	24	82.5	-	7.5	-	90
	A. flavus var columnaris	25	19.5	-	-	6.4	25.9
	A. flavus var columnaris	26	-	-	-	-	-
Cardamom	*A. flavus*	27	40.2	30.8	-	-	71
	A. flavus	28	18.3	1.1	15.9	14.1	49.4
	A. flavus	29	-	-	2.45	-	2.45
	A. flavus	30	-	-	-	-	-
	A. flavus	31	-	-	-	-	-
	A. flavus	32	-	-	-	-	-

Medicinal plants	Fungal isolates	Isolate No.	Aflatoxins concentrations (ng/ml)				
			B1	B2	G1	G2	Total
Carob	*A. flavus var columnaris*	33	-	2.3	-	15.8	8.1
	A. flavus var columnaris	34	-	-	-	-	-
Castor beans	*A. flavus var columnaris*	35	20.1	4.2	1.5	8.1	33.9
	A. flavus var columnaris	36	-	-	-	-	-
	A. flavus var columnaris	37	23.2	2	2.8	10.3	38.3
Coriander	*A. parasiticus*	38	-	59.2	29.8	11.5	100.5
Cress seeds	*A. flavus var columnaris*	39	-	-	-	-	-
Cumin	*A. flavus*	40	-	-	-	-	-
	A. flavus	41	-	-	-	-	-
Fennel	*A. flavus var columnaris*	42	3.5	-	-	-	3.5
	A. flavus var columnaris	43	-	-	18.8	-	18.8
	A. flavus var columnaris	44	-	-	10.8	-	10.8
Fenugreek	*A. flavus var columnaris*	45	35	-	-	11	46
	A. flavus	46	28.2	-	-	0.1	28.3
	A. flavus var columnaris	47	-	-	-	-	-
Ginger	*A. flavus var columnaris*	48	195.5	-	-	-	195.5
	A. parasiticus	49	100.2	7.6	-	-	107.8
	A. flavus var columnaris	50	-	-	-	-	-
	A. flavus var columnaris	51	-	-	-	-	-
	A. flavus var columnaris	52	-	-	-	-	-
	A. flavus var columnaris	53	-	-	-	-	-
	A. flavus var columnaris	54	-	-	-	-	-
	A. flavus var columnaris	55	17.5	2.8	12.5	7	39.8
	A. flavus var columnaris	56	22	0.3	18	4.5	44.8
	A. flavus var columnaris	57	-	-	-	-	-
	A. flavus var columnaris	58	-	-	-	-	-

Table 4. Continue

Medicinal plants	Fungal isolates	Isolate No.	Aflatoxins concentrations (ng/ml)				
			B1	B2	G1	G2	Total
Ginger	*A. flavus var columnaris*	59	-	-	-	-	-
	A. flavus var columnaris	60	-	-	-	-	-
	A. flavus var columnaris	61	-	-	-	-	-
	A. flavus var columnaris	62	-	-	-	0.1	0.1
	A. flavus var columnaris	63	-	-	-	-	-
	A. flavus var columnaris	64	10	-	24	-	34
	A. flavus var columnaris	65	-	-	-	-	-
	A. flavus var columnaris	66	-	-	-	-	-
	A. flavus var columnaris	67	-	-	-	-	-
	A. parasiticus	68	58	2.2	-	-	60.2
	A. flavus var columnaris	69	-	-	-	-	-
	A. flavus var columnaris	70	-	-	-	-	-
	A. flavus var columnaris	71	-	-	-	-	-
	A. flavus var columnaris	72	-	-	-	-	-
	A. flavus var columnaris	73	-	-	-	-	-
	A. flavus var columnaris	74	-	-	-	-	-
	A. flavus var columnaris	75	-	-	-	-	-
	A. flavus var columnaris	76	34	-	18.3	0.1	52.4
	A. flavus var columnaris	77	-	-	-	-	-
	A. flavus var columnaris	78	-	-	-	-	-
Karkade	*A. flavus*	79	-	-	-	-	-
Linseeds	*A. flavus*	80	48.3	5.8	9.5	35	98.6
	A. flavus var columnaris	81	-	-	-	-	-
	A. flavus	82	30.8	4.4	10.1	23.1	68.4
Liquorice	*A. flavus*	83	0.23	-	-	-	0.23
	A. flavus	84	-	-	-	-	-
Majoram	*A. flavus var columnaris*	85	-	-	-	0.75	0.75
	A. flavus var columnaris	86	-	-	-	-	-
	A. flavus var columnaris	87	-	-	-	-	-
	A. flavus var columnaris	88	-	-	4.45	-	4.45

Medicinal plants	Fungal isolates	Isolate No.	Aflatoxins concentrations (ng/ml)				
			B1	B2	G1	G2	Total
Moghat	*A. flavus*	89	118.8	2.5	7.8	-	129.1
	A. flavus	90	59.8	10	1.9	-	71.7
	A. flavus	91	-	-	-	-	-
	A. flavus	92	-	-	-	-	-
	A. flavus var columnaris	93	-	-	-	-	-
	A. flavus var columnaris	94	-	-	-	-	-
	A. flavus var columnaris	95	-	-	-	-	-
	A. flavus var columnaris	96	-	-	-	-	-
	A. flavus var columnaris	97	-	-	-	-	-
	A. flavus var columnaris	98	-	-	-	-	-
Mustard	*A. flavus var columnaris*	99	30	0.1	12	12.4	54.5
	A. parasiticus	100	22.1	2.2	10.5	15.8	50.6
	A. flavus var columnaris	101	-	-	-	-	-
	A. flavus var columnaris	102	-	-	-	-	-
Nigella	*A. flavus var columnaris*	103	1.7	-	4.5	-	6.2
	A. flavus var columnaris	104	2.5	-	-	0.9	3.4
	A. flavus var columnaris	105	0.9	-	5.2	-	6.1
	A. flavus	106	1.8	-	6.6	-	8.4
	A. flavus var columnaris	107	-	-	0.23	-	0.23
	A. flavus var columnaris	108	-	-	-	-	-
	A. flavus	109	-	-	-	-	-
	A. flavus var columnaris	110	-	-	-	-	-
Nutmeg	*A. parasiticus*	111	624.95	-	91.2	29.7	745.9
	A. parasiticus	112	27.8	-	36.2	13.9	77.9
	A. parasiticus	113	311.7	-	300.1	36.6	648.4
Parsley	*A. flavus var columnaris*	114	-	19.95	185.4	116.3	321.7
	A. flavus var columnaris	115	58.5	9.8	177.5	86.7	332.5
	A. flavus var columnaris	116	227.2	7.5	59.8	-	294.5

Table 4. Continue

Parsley	*A. flavus var columnaris*	117	36.7	-	14.4	-	51.1
Radish	*A. flavus var columnaris*	118	-	-	-	-	-
	A. flavus	119	-	-	-	-	-
	A. flavus var columnaris	120	-	-	103.4	57.7	161.1
	A. flavus	121	51.7	13.99	32.9	42.2	140.8
Rocket	*A. flavus var columnaris*	122	187.5	381.8	-	41	610.3
	A. flavus	123	-	-	-	-	-
Rosemary	*A. flavus var columnaris*	124	7.9	-	5.5	-	13.4
	A. flavus var columnaris	125	-	-	-	-	-
	A. flavus var columnaris	126	8.9	-	-	-	8.9
	A. flavus var columnaris	127	-	-	-	-	-
Sage	*A. flavus*	128	-	-	5.54	-	5.54
	A. flavus	129	-	-	-	-	-
Santonica	*A. flavus var columnaris*	130	1	-	9.8	-	10.8
	A. flavus var columnaris	131	12.1	-	-	5.7	17.8
	A. flavus var columnaris	132	-	-	-	-	-
	A. flavus	133	10.5	-	1.2	-	11.7
	A. flavus	134	-	-	-	-	-
Senna	*A. flavus var columnaris*	135	796.1	0.19	16.3	5.6	818.19
	A. flavus var columnaris	136	268.2	2.2	70.1	7.6	348.1
	A. flavus	137	-	-	-	-	-
	A. flavus	138	-	-	-	-	-
	A. flavus	139	-	-	-	-	-
	A. flavus var columnaris	140	-	-	145.5	52.5	198
	A. flavus	141	-	-	-	-	-
	A. flavus var columnaris	142	-	-	-	-	-
	A. flavus	143	22.5	2.5	6.8	-	31.8
	A. flavus var columnaris	144	-	-	-	-	-
	A. flavus var columnaris	145	-	-	-	-	-
	A. flavus	146	-	-	-	-	-
	A. flavus	147	45.3	-	34	-	79.3
	A. flavus var columnaris	148	-	-	-	-	-
	A. flavus var columnaris	149	-	-	-	-	-
Thyme	*A. flavus*	150	-	-	-	-	-
Turmeric	*A. parasiticus*	151	-	-	1	-	1

Table 5. Aflatoxins occurrence in some medicinal plants

Medicinal plants	Aflatoxins concentrations (ng/g)				
	B1	B2	G1	G2	Total
Ammi	-	-	724.6	-	724.6
Anise	1.98	-	157.5	-	159.48
Black pepper	75	-	31.5	16	122.5
Cacao	-	-	-	-	-
Cardamom	-	-	-	-	-
Carob	-	-	-	-	-
Castor beans	15.5	-	13.8	-	29.3
Coriander	-	-	-	-	-
Cress seeds	-	-	-	-	-
Cumin	-	-	-	-	-
Fennel	-	-	-	-	-
Fenugreek	-	-	2	-	2
Ginger	26.9	-	-	-	26.9
Karkade	-	-	-	-	-
Linseeds	25.5	-	13.3	-	38.8
Liquorice	57	-	1.5	-	58.5
Majoram	3.25	-	-	-	3.25
Moghat	-	-	4	-	4
Mustard	18.2	-	10.5	7.6	36.3
Nigella	56.8	1.25	4.4	0.25	62.7
Nutmeg	-	-	-	-	-
Parsley	27.4	2.5	8.1	-	38
Radish	-	-	-	-	-
Rocket	19.9	-	5.5	-	25.4
Rosemary	3.1	-	12.9	-	16
Sage	-	-	6.25	-	6.25
Santonica	1.4	-	0.1	-	1.5
Senna	-	30.5	-	-	30.5
Thyme	16.8	-	41.2	-	57.9
Turmeric	-	-	-	-	-

The results obtained in Table (4) showed that, thirteen isolates from different types of substrates have the ability to produce four types of afltoxins (B_1, B_2, G_1, G_2); these were: *A. flavus var columnaris* (No. 35, 37, 55, 56, 99, 115, 135 & 136), *A. flavus* (No. 28, 80, 82 & 121) and *A. parasiticus* (No.100). Eleven isolates produced 3 types of aflatoxins, of which *A. flavus var columnaris* (No. 76) and *A. parasiticus* (No. 111, 112 & 113) produced B1,G1 & G2, *A. flavus var columnaris* (No. 116) and *A. flavus* (No. 89, 90 & 143) produced B1, B2 & G1 while *A. parasiticus* (No. 38) and *A. flavus var columnaris* (No.114) produced B2, G1& G2 and *A. flavus var columnaris* (No. 122) produced B1, B2 & G2. The remaining isolates produced one or two types of aflatoxins. In this respect, [45] found that 42.9% *A. flavus* isolates were found to be aflatoxigenic strains and indicated that 27.6% of isolates have the ability to produce aflatoxin B_1 or aflatoxins B_1 and B_2; 45.5% *Aspergillus parasiticus* have the ability to produce aflatoxins B_1, B_2, G_1 and G_2 from herbal drugs.

As illustrated in Table (4), the most active aflatoxins produceing isolates were *A. flavus var columnaris* (No. 135) isolated from senna, which produced 796.1, 0.19, 16.3 & 5.6 ng/ml from aflatoxins B1, B2, G1 &G2 respectively, *A. flavus* (No. 20) isolated from black pepper, which produced 768.3 & 22.6 ng/ml from aflatoxins B1& G1 respectively, *A. parasiticus* (No. 111) isolated from nutmeg which produced 624.95, 91.2 & 29.7 ng/ml from aflatoxins B_1, G_1 & G_2 respectively, *A. parasiticus* (No. 113) isolated from nutmeg which produced 311.7, 300.1 & 36.6 ng/ml from aflatoxins B_1, G_1 & G_2 respectively and *A. flavus var columnaris* (No. 122) isolated from rocket which produced 187.5, 381.8 & 41 ng/ml from aflatoxins B_1, B_2 & G_2 respectively. On the other hand, the least active aflatoxins producers were *A. flavus var columnaris* (No. 62) isolated from ginger which produced 0.1ng/ml from aflatoxin G_2, *A. flavus var columnaris* (No. 107) isolated from nigella produced aflatoxin G_1 & *A. flavus* (No. 83) isolated from liquorice produced aflatoxin B_1 with concentration of 0.23 ng/ml for each and *A. flavus var columnaris* (No. 85) isolated from majoram which produced 0.75 ng/ml from aflatoxin G_2. [54] reported that all *Aspergillus flavus* from cardamom and black pepper

produced aflatoxin B_1 in amounts ranging from 65 to 3000 ng/ml. [34] isolated twenty *Aspergillus flavus* strains from ginger, black pepper, and cinnamon and were screened for aflatoxins production. Nine isolates (45%) were found to produce aflatoxin B_1, at a level ranging between 50 $\mu g/l^{-1}$ and 90 $\mu g/l^{-1}$. Almost similar observations were reported by [33,46,55].

3.3 Occurrence of Aflatoxins in Medicinal Plant Samples

The results in Table (5) revealed that of 30 medicinal plant samples screened, 19 samples (63.3%) were found contaminated with aflatoxins in the range of 1.5 to 724.6 ng/g. Nigella was the only substrate which contained the four types of aflatoxins (B_1, B_2, G_1 & G_2) while parsley, black pepper and mustard contained 3 types of aflatoxins which were (B_1, G_1 & G_2) for black pepper and mustard and (B_1, G_1 & B_2) for parsley. The remaining substrates contained one or two types of aflatoxins.

As shown in Table (5), the highest levels of aflatoxins contamination were recorded in ammi (724.6 ng/g), anise (159.5 ng/g) and black pepper (122.5 ng/g) while the lowest levels were detected in majoram (3.25 ng/g), fenugreek (2 ng/g) and santonica (1.5 ng/g). These results were in agreement with those reported by [56] revealed that aflatoxin B_1 was found in 41 out of 93 spice samples with levels ranging from 4.9-8.4 µg/kg in anise samples and 24.6-30 µg/kg in case of black pepper samples. Also, [46] found that the highest aflatoxins contents were detected in red chili, coriander and black pepper with concentrations of 219.6, 179.5 and 185 ng/g respectively. Similar results were obtained by [55,57-60].

The presence of aflatoxins in medicinal plants has numerous public health effects because these toxins causes liver cancer in certain animals and also humans can suffer acute liver damage from ingestion of high amount of aflatoxins Jeswal P, Kumar D [46]. Therefore, it is necessary to further investigate the presence of aflatoxins in these commodities. Plant materials, designed for medical uses, should be carefully stored and evaluated for aflatoxins presence before use inorder to ensure they are safe for consumers. Moreover, good agricultural practices should be performed to reduce the presence of moulds on the medicinal plants and thus decrease the possibility of aflatoxins production.

4. CONCLUSION

The use of medicinal plants as the first choice in self-treatment of minor conditions continues to expand rapidly across the world. This makes the safety of these plants an importanat public health issue. This study has shown that medicinal plants are prone to contamination by moulds including toxigenic *Aspergillus* and although aflatoxins levels may be low, the risk of aflatoxicosis resulting from the continuous ingestion of these foods may be high. The presence of *Aspergillus* and hence aflatoxins contamination in the samples may be due to poor storage, transport and handling conditions in the local market. The findings from this study also repeat the need for constant quality assessment of medicinal plants in the market in order to ensure that medicinal plant materials and products are suitable for human consumption. Medicinal plants sold in markets should be placed in clean, sterile baskets or suitable hygienic packs. The moisture content of the plant materials should always be maintained at minimal levels to lower the rate of fungal propagation. Moreover, studies on human exposure and risk assessment of aflatoxins in medicinal plants are needed to be carried out for protecting consumers from adverse effects associated with aflatoxins contaminated medicinal plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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